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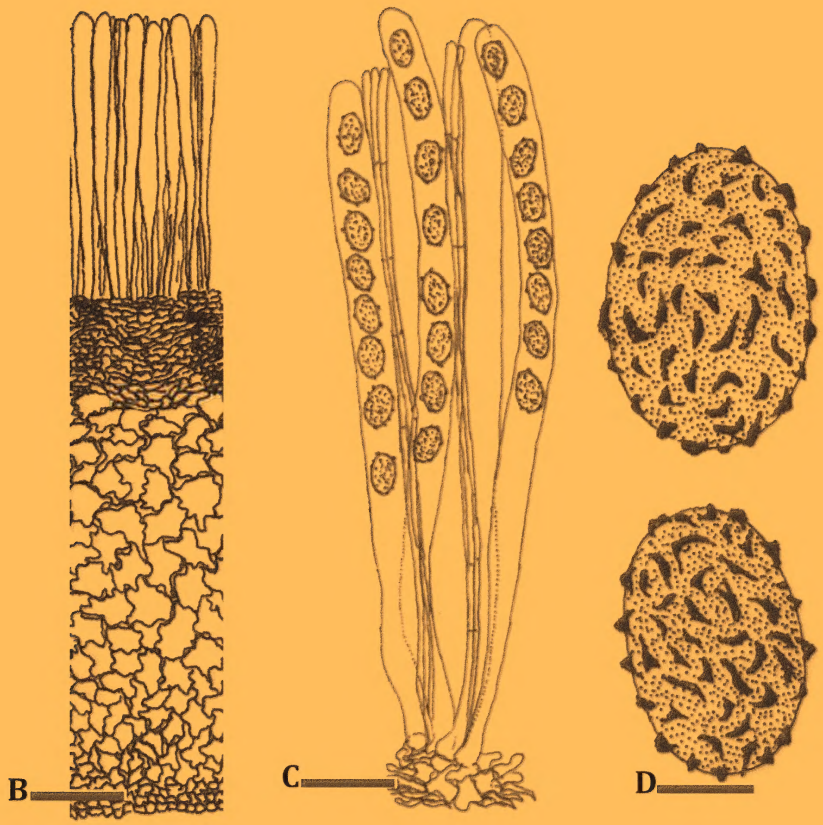

Peziza succosa in Pakistan
(Ashraf & Khalid— PLATE 2, p. 304)
TAYIBA ASHRAF, *artist*

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VOLUME ONE HUNDRED NINETEEN — TABLE OF CONTENTS

Cover section

Errata viii
Reviewers ix
Submission procedures x
From the Editor xi

Research articles

Four new species of *Diorygma* from India
Bharati Sharma & Pradnya Khadilkar 1
Gyalidea minuta in Central Europe — new data on its distribution, ecology, and morphological variation Dariusz Kubiak & Jiří Malíček 11
New species and record of *Sporidesmium* from southern China
Jian Ma, Li-Guo Ma, Yi-Dong Zhang, Ji-Wen Xia & Xiu-Guo Zhang 17
Phlebopus spongiosus sp. nov. (*Boletales, Boletinellaceae*) with a sponge-like tissue Nguyen Duc Hoang Pham, Haruki Takahashi, Toshimitsu Fukiharu, Kiminori Shimizu, Ba Dung Le & Akira Suzuki 27
Type studies in *Helvella* (*Pezizales*) 1
Fidel Landeros, Teresa Iturriaga & Laura Guzmán-Dávalos 35
Psilocybe s.s. in Thailand: four new species and a review of previously recorded species Gastón Guzmán, Florencia Ramírez Guillén, Kevin D. Hyde & Samantha C. Karunarathna 65
A new *Corynesporella* species and two first records from China
Li-Guo Ma, Jian Ma, Yi-Dong Zhang & Xiu-Guo Zhang 83
Queletia mirabilis, a rare gasteroid species in Gran Canaria, Spain
G. Moreno, M.A. Ribes, V. Escobio & A.N. Khalid 89
New species and a new record of *Solicorynespora* from southern China
Jian Ma, Li-Guo Ma, Yi-Dong Zhang, Ji-Wen Xia & Xiu-Guo Zhang 95
Two new species of *Endophragmiella* from southern China
Jian Ma, Yi-Dong Zhang, Li-Guo Ma, Rafael F. Castañeda-Ruiz & Xiu-Guo Zhang 103
Alveariospora, a new anamorphic genus from trichomes of *Dimorphandra mollis* in Brazil Meiriele da Silva, Rafael F. Castañeda-Ruiz, Olinto Liparini Pereira & Robert Weingart Barreto 109

Intraornatosporaceae (*Gigasporales*), a new family with two new genera and two new species Bruno T. Goto, Gladstone A. Silva, Daniele M.A. de Assis, Danielle K.A. Silva, Renata G. Souza, Araeska C.A. Ferreira, Khadija Jobim, Catarina M.A. Mello, Helder E.E. Vieira, Leonor C. Maia & Fritz Oehl 117

Radulomycetopsis (*Agaricomycetes*), a new corticioid genus from India G.S. Dhingra, Priyanka & Jaspreet Kaur 133

First record of *Dendrothele mangiferae* (*Agaricales*, *Basidiomycota*) from the Neotropics Sergio P. Gorjón 137

Two new fungi from Mexico: *Anaseptoidium* gen. nov. and *Cylindrosympodium sosae* sp. nov. Rafael F. Castañeda-Ruiz, Gabriela Heredia, Rosa M. Arias-Mota, Marc Stadler, Masatoshi Saikawa & Eric H.C. McKenzie 141

Candelariella, Ochrolechia, Physcia, and *Xanthoria* species new to Turkey Kenan Yazıcı, André Aptroot & Ali Aslan 149

A new species and new combinations and records of *Hypotrachyna* and *Remototrachyna* from Bolivia Adam Flakus, Pamela Rodriguez Saavedra & Martin Kukwa 157

Mycena moconensis, a new species in section *Polyadelphia* from Argentina N. Niveiro, O. Popoff, D. Desjardin & E. Albertó 167

A new species of *Fulvifomes* (*Hymenochaetaceae*) from Cambodia Li-Wei Zhou & Wei-Min Zhang 175

Fungal pathogens of 'cat's claws' from Brazil for biocontrol of *Macfadyena unguis-cati* Meiriele da Silva, Robert W. Barreto & Olinto Liparini Pereira 181

Typification of *Lecanora expallens* and *L. expallens* var. *conizaea* (*Lecanorales*, *Ascomycota*) Martin Kukwa 197

The morphology of *Ganoderma* species with a laccate surface Mabel Gisela Torres-Torres & Laura Guzmán-Dávalos 201

Four new polypore species from the western United States Josef Vlasák, Josef Vlasák Jr. & Leif Ryvarden 217

Studies on Croatian *Basidiomycota* 2: *Marasmiellus milicae* sp. nov. Armin Mešić, Zdenko Tkalčec & Vladimír Antonín 233

Zelodactylaria, an interesting new genus from semi-arid northeast Brazil Alisson Cardoso Rodrigues Da Cruz, Luís Fernando Pascholati Gusmão, Rafael F. Castañeda-Ruiz, Marc Stadler & David W. Minter 241

A new species of *Ijuhya, I. oenanthicola* Christian Lechat & Michel Hairaud 249

Two new species of *Hyphoderma* (*Agaricomycetes*) from India
Priyanka & G.S. Dhingra 255
New records of noteworthy gasteroid fungi from Pakistan
N. Yousaf, A.R. Niazi & A.N. Khalid 261
Pseudocercosporella stellariicola, a new hyphomycete occurring on *Stellaria aquatica* Mi-Jeong Park, Ji-Hyun Park & Hyeon-Dong Shin 269
A key to the peltigericolous fungi in Turkey
Mehmet Gökhan Halici, Mehmet Candan & Ayşen Türk 277
Taxonomy of the *Gymnopus inusitatus* group and the new *G. inusitatus* var. *cystidiatus* from Hungary Vladimír Antonín, Péter Finy & Michal Tomšovský 291
New records of *Pezizales* from Pakistan T. Ashraf & A.N. Khalid 301
A new *Hysterostomella* species from the Cerrado in Brasília National Park C.A. Inácio, R.C. Pereira-Carvalho, E.S.C. Souza, H.B. Sales & J.C. Dianese 307
Penicillium mallochii and *P. guanacastense*, two new species isolated from Costa Rican caterpillars Karol G. Rivera, Joel Díaz, Felipe Chavarría-Díaz, Maria Garcia, Mirjam Urb, R. Greg Thorn, Gerry Louis-Seize, Daniel H. Janzen & Keith A. Seifert 315
Two new species of *Terriera* from Yunnan Province, China
Juan-fang Song, Ling Liu, Yuan-yuan Li & Cheng-Lin Hou 329
Gloeocystidiellum kenyense in Azores and Madeira M. Teresa Telleria, Margarita Dueñas, Ireneia Melo, Esperanza Beltrán-Tejera, J. Laura Rodríguez-Armas, Isabel Salcedo & María P. Martín 337
A new species of *Skeletocutis* on bamboo (*Polyporaceae*) in tropical China Li-Wei Zhou & Wen-Min Qin 345
Paxillus albidulus, P. ammoniavirescens, and *P. validus* revisited
Else C. Vellinga, Erin P. Blanchard, Stephen Kelly & Marco Contu 351
Two new *Heteroconium* species and two other forest microfungi newly recorded from China Shou-Cai Ren, Jian Ma & Xiu-Guo Zhang 361
Second record of *Ramularia hypericicola* — collected in Turkey on a new host Faruk Selçuk, Elşad Hüseyin & Ali S. Bulbul 369
Lichenological notes 4: A Revision of *Acarospora gallica* (*Acarosporaceae*) Kerry Knudsen & Jana Kocourková 373
The genus *Xylaria* in the south of China - 3. *Xylaria atroglobosa* sp. nov. Hai-Xia Ma, Larissa Vasilyeva & Yu Li 381
A new name for a *Coltricia* (*Basidiomycota*) from India
Juliano M. Baltazar & Rosa Mara B. da Silveira 385
Tuber in China: *Tuber microspermum* and *T. microspiculatum* spp. nov.
Li Fan, Jin-Zhong Cao, Zhao-Hui Zheng, & Yu Li 391

The first report of *Ceriporia lacerata* (*Phanerochaetaceae, Basidiomycota*) in Korea — Yeongseon Jang, Ha Eun Choi, Young Woon Lim, Jin Sung Lee & Jae-Jin Kim 397

Marasmioid and gymnopoid fungi of the Republic of Korea. 5. *Marasmius* sect. *Hygrometrici* — Vladimír Antonín, Rhim Ryoo, Kang-Hyeon Ka & Hyeon-Dong Shin 405

A new hairy species of *Leptogium* (*Collemataceae*) from China — Hua-Jie Liu & Shuai Guan 413

Abrachium, a new genus in the *Clathraceae,* and *Itajahya* reassessed — Tiara Sousa Cabral, Paulo Marinho, Bruno Tomio Goto & Iuri Goulart Baseia 419

Nomenclatural notes 13. An incorrect neotype designation and provision for a lectotype and an epitype for *Helvella fusca* — Fidel Landeros & Richard P. Korf 431

Herpothallon weii, a new lichen from China — Yu-Liang Cheng, Jing Ning, Hou-Ping Xu, Lu-Lu Zhang, Hai-Ying Wang & Zun-Tian Zhao 439

Four new records of lecideoid lichens from China — Lu-Lu Zhang, Li-Song Wang, Hai-Ying Wang & Zun-Tian Zhao 445

Puccinia, Uromyces, and *Xenodochus* species new to Turkey — Sanli Kabaktepe & Zeliha Bahçecioğlu 453

Pseudobaeospora deckeri sp. nov., a new agaric from central California — Christian Schwarz 459

A new species of *Volvariella* from India — Gunasekaran Senthilarasu, Rahul Sharma & Sanjay K. Singh 467

A new species of *Pleurophragmium* from India — Maria A. D'Souza & D.J. Bhat 477

New combinations in *Lactifluus*. 2. *L.* subgenus *Gerardii* — D. Stubbe, X.-H. Wang & A. Verbeken 483

Book reviews and notices — Else C. Vellinga (Editor) 487

Mycobiotas online 493

Abstracts: Niveiro & Albertó—Checklist of the Argentine *Agaricales*. I; Costa & al.—Checklist of endophytic fungi from tropical regions; Nováková et al.—List of cultivable microfungi...of the Czech and Slovak Republics; Ghobad-Nejhad & Hallenberg—Checklist of Iranian non-gilled/non-gasteroid hymenomycetes (*Agaricomycotina*); Bahçecioğlu & Kabaktepe—Checklist of rust fungi in Turkey

Nomenclature

Minimizing the chaos following the loss of Article 59: Suggestions for a discussion — Walter Gams, Richard A. Humber, Walter Jaklitsch, Roland Kirschner & Marc Stadler 495

Nomenclatural novelties proposed in volume 119 509

Errata from previous volumes

Volume 118

p. 248, Figure caption, line 2

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With tight budgeting and loyal subscribers, for 38 years Mycotaxon has been able to support its authors without asking for their help. We must now make a change. I am truly sorry to announce that, due to reduced revenues, Mycotaxon will assess charges of $20 per published page for all manuscripts submitted after 1 April 2012.

When we moved to online publication last year, we knew that the change would affect our revenues. However, as shelf space declined in libraries and postage costs soared, we had little choice. After transition glitches, online publication is now working well and files are downloaded quickly with little problem. However, a review of 2011 expenditures and revenue by our Business Manager revealed that Mycotaxon has slipped from a 'break-even' point to a serious deficit.

After considerable discussion of available alternatives, we have concluded that the best option is to assess authors a modest page charge rather than increase subscription rates and lose additional subscribers. To preserve subscriptions, open access will continue to be available only for an additional optional fee (instead of granting each paper automatic open access — our philosophical preference).

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Mycotaxon has always operated on a very low cost basis. The new charges are intended only to cover anticipated expenses and bring us back to a break-even point. We believe that Mycotaxon provides a valuable service to authors as well as readers and mycology as a whole. We hope our authors will share this view and continue their submissions.

With warm regards and hopes for a solvent future,
Lorelei Norvell, Mycotaxon *Editor-in-Chief*
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Four new species of *Diorygma* from India

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Abstract—Four new species of the lichen genus *Diorygma* from India are described — *D. agumbense, D. dandeliense,* and *D. karnatakense* from the Western Ghats and *D. rupicola* from Northeast India.

Keywords—ascomycetes, taxonomy, *Ostropales, Graphidaceae*

Introduction

The lichen genus *Diorygma* Eschw. is widespread in tropical and subtropical regions and c. 45 species are known from around the world (Kalb et al. 2004, Archer 2006, 2007, Archer & Elix 2008, Cáceres 2007, Sharma & Makhija 2009a,b, Makhija et al. 2009, Tripp et al. 2010), 21 of which are known from India. The main characters that define *Diorygma* are lirellate ascocarps with a heavily pruinose disc, parallel paraphyses with thin lumina, thick gelatinous walls, and tips that anastomose to form an epithecium, *Graphis*-type asci, and hyaline to (rarely) brownish trans-septate or muriform ascospores. Kalb et al. (2004), who monographed the genus, and confirmed its placement within *Graphidaceae* through molecular phylogenetic analysis.

The combination of norstictic and salazinic acids is very rare in *Diorygma*. In our previous studies on *Graphidaceae* from India, we proposed four new *Diorygma* species with norstictic and salazinic acids as major compounds: *D. dealbatum* B.O. Sharma & Makhija, *D. inaequale* B.O. Sharma & Makhija, *D. manipurense* B.O. Sharma & Makhija, and *D. verrucirimosum* B.O. Sharma & Makhija (Sharma & Makhija 2009a). Since then, we have encountered four additional new species of *Diorygma,* which are described here. Two — *D. dandeliense* and *D. karnatakense* — contain only norstictic and salazinic acids, while *D. agumbense* (with protocetraric acid) and the saxicolous *D. rupicola* are also new to science. A key to the identification of *Diorygma* species in India is provided.

Materials & methods

Approximately 80 specimens representing four *Diorygma* species were collected from different regions of India Western Ghats —Karnataka, Northeast India —Meghalaya. Their external morphology was studied using a Zeiss dissecting microscope; sections for anatomical studies were stained with Lugol's iodine, mounted in lactophenol, and examined with a Zeiss Axioskop microscope. Images were captured using a Nikon Coolpix 8400 digital camera.

The specimens were identified by comparison with protologues or descriptions and photographs of known species in the most recent treatments (Kalb et al. 2004, Archer 2006, 2007, Archer & Elix 2008, Makhija et al. 2009; Sharma & Makhija 2009a,b).

Chemical constituents were identified by thin-layer chromatography using methods standardized for lichen products (Culberson & Kristinsson 1970, Culberson 1972, White & James 1985) with the solvent systems benzene-dioxane-acetic acid (180:45:5), hexane-ethyl ether-formic acid (130:80:20), and toluene-ethyl acetate-formic acid (139:83:8). All examined specimens are deposited in Ajrekar Mycological Herbarium (AMH).

Taxonomy

Diorygma agumbense B.O. Sharma & Khadilkar, **sp. nov.** FIG. 1

MycoBank MB 561545

Species nova distincte inter Diorygma *specibus ob continentem acidum protocetraricum sed ascosporis 8-nae differt.*

TYPE: India. Karnataka: Hebri, Agumbe-Udipi road, 16.12.1974, Patwardhan & A.V. Prabhu (**Holotype**, AMH 74.3041).

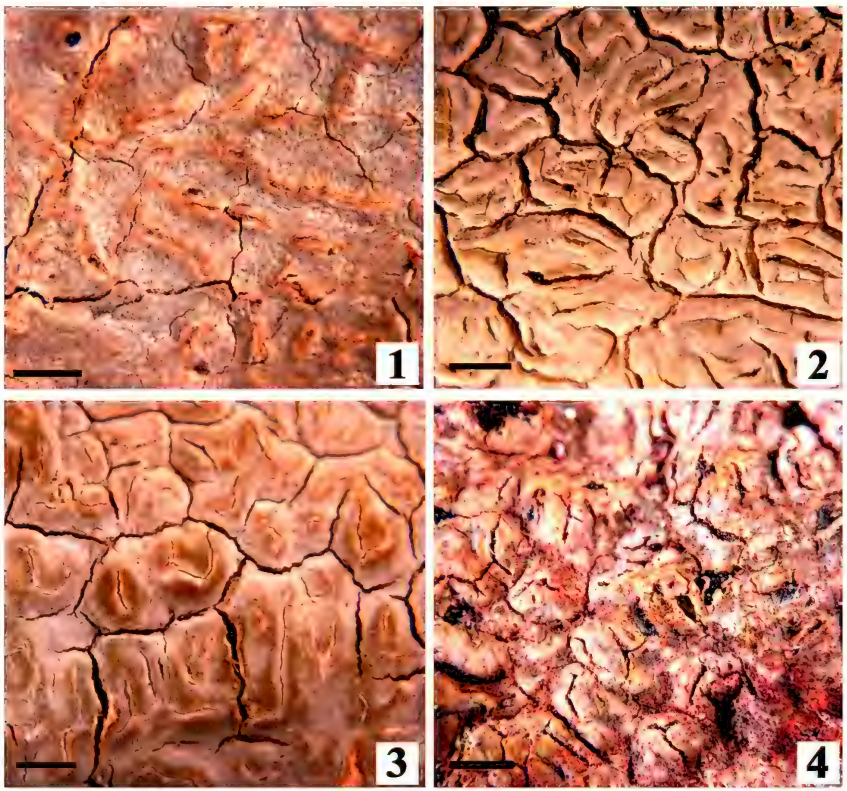
ETYMOLOGY: Referring to Agumbe, the collecting locality

Thallus corticolous, pale greenish-grey, rough, cracked, delimited by thin, black hypothallus. Ascocarps lirelline, concolorous with the thallus, 1–1.5 mm long, mostly simple, rarely branched, slightly raised to mostly immersed. Disc narrow, slit-like, rarely ± open, covered with white pruina. Exciple convergent, non-carbonized, non-striate. Hymenium hyaline, laterally KI+ blue, clear, not inspersed. Asci 8-spored, hyaline, muriform, I+ blue violet, peripheral and central locules of more or less equal size, regularly arranged in rows, 87–125 × 20–37 µm.

CHEMISTRY—Cryptostictic, protocetraric, and stictic acids present (K+ yellow, P+ red).

ECOLOGY & DISTRIBUTION— in semi-evergreen forest in open places on the roadsides. Known only from the type locality.

REMARKS—*Diorygma africanum* Kalb et al., *D. hololeucum* (Mont.) Kalb et al., and *D. pruinosum* (Eschw.) Kalb et al., the only other *Diorygma* species known to have protocetraric acid as a major compound, differ in having a distinctly divergent exciple, 1–2-spored asci, and an absence of stictic acid. The combination of protocetraric and stictic acid complex is rather unique in the genus and no species previously has been reported with this chemistry.



FIGURES 1–4. Habit: 1. *Diorygma agumbense* (Holotype AMH 74.3041); 2. *D. dandeliense* (Holotype AMH 04.276); 3. *D. karnatakense* (Holotype AMH 04.280); 4. *D. rupicola* (Holotype AMH 77.779). Scale bars = 1 mm.

Diorygma erythrellum (Mont.) Kalb et al. and *D. minisporum* Kalb et al. both have 8-spored asci but differ from *D. agumbense* in small ascospores and chemistry. *Diorygma erythrellum* has ascospores of 30–65 × 12–20 µm and produces norstictic acid, whereas *D. minisporum* has transversely septate ascospores of 17–20 × 5–6.5 µm and lacks lichen substances.

Diorygma dandeliense B.O. Sharma & Khadilkar, **sp. nov.** FIG. 2

MYCOBANK MB 561546

Similis Diorygma dealbatum *sed ascospois majoribus et excipulum convergentum differt.*

TYPE: India, Karnataka, Dandeli forest, 5.10.2004, U.V. Makhija (**Holotype**, AMH 04.276).

ETYMOLOGY: Referring to Dandeli, the collecting locality

Thallus corticolous, pale greenish-grey, smooth. Ascocarps lirelline, concolorous with the thallus, 0.5–2 mm long, mostly simple, rarely branched, curved, slightly raised to immersed. Disc narrow, slit-like, rarely ± open, covered with white pruina. Exciple convergent, non-carbonized, non-striate. Hymenium hyaline, laterally KI+ blue, clear, not inspersed. Asci 1-spored, hyaline, muriform, I+ blue violet, peripheral and central locules of more or less equal size, regularly arranged in rows, 125–175 × 27–35 µm.

Chemistry—Norstictic and salazinic acids present (K+ yellow forming red crystals).

Additional specimens examined—**INDIA. Karnataka:** Dandeli forest, 5.10.2004, U.V. Makhija, AMH 04.277; B.C. Behera, AMH 04.269.

Remarks—*Diorygma dandeliense* shares a similar morphology and chemistry with *D. dealbatum,* which can be differentiated by its shorter (105–147 µm) ascospores and a divergent exciple.

The new species is very similar to *D. megasporum* Kalb et al. in morphology and ascospore range. However, the asci of *D. megasporum* contain 2–6 ascospores and the thallus lacks salazinic acid.

Both *D. excipuloconvergentum* Makhija et al. and *D. albocinerascens* Makhija et al., which are morphologically and chemically very close to *D. dandeliense,* have long (≤ 6 mm) lirellae, distinctly striate exciple, and 1–2-spored asci.

Diorygma salvadoriense Kalb et al. also contains norstictic and salazinic acids but differs from the new species in having distinctly divergent exciple and a carbonaceous basal layer.

Diorygma karnatakense B.O. Sharma & Khadilkar, **sp. nov.** Fig. 3

MycoBank MB 561547

Similis Diorygma salvadoriense *sed peripheralis et centralis sporae loculis equalis differt.*

Type: India, Karnataka, Dandeli forest, 5.10.2004, U.V. Makhija (**Holotype**, AMH 04.280).

Etymology: From the state name, Karnataka.

Thallus corticolous, epiphloeodal, greenish-grey, dull, cracked, smooth. Ascocarps lirelline, concolorous with the thallus, 0.5–2 mm long, simple, curved, immersed, ends acute or rounded. Disc narrow, slit-like, covered with white pruina. Exciple convergent, non-carbonized, non striate. Hymenium hyaline, laterally KI+ blue, clear, not inspersed. Asci 1–4-spored, hyaline, muriform, I+ blue violet, peripheral and central locules of more or less equal size, 130–200 × 32–42 µm.

Chemistry— Norstictic and salazinic acids present (K+ yellow, forming red crystals).

Additional specimens examined—**INDIA. Karnataka:** Dandeli forest, 5.10.2004, U.V. Makhija, AMH 04.266, 04.441, 04.291.

Remarks—*Diorygma karnatakense* is close to *D. salvadoriense, D. reniforme* (Fée) Kalb et al., *D. rufopruinosum* (A.W. Archer) Kalb et al., *D. albocinerascens,* and *D. excipuloconvergentum* in having large ascospores and in containing norstictic and salazinic acids as major substances. However, *D. salvadoriense, D. rufopruinosum,* and *D. reniforme* do not produce ascospores with the same-sized peripheral and central cells. Moreover, *D. salvadoriense* and *D. reniforme* have a distinctly divergent, orange brown to basally carbonized, entire exciple.

Diorygma albocinerascens and *D. excipuloconvergentum* from Maharashtra, India, also containing norstictic and salazinic acids and morphologically similar, differ from *D. karnatakense* in a distinctly striate exciple.

Diorygma rupicola B.O. Sharma & Makhija, **sp. nov.** Fig. 4

MycoBank MB 561548

Similis Diorygma hieroglyphicum *sed habitus saxicola et excipulum convergentum differt.*

Type: India, Meghalaya, Mawrong, on Cherrapunjee Road, 27.10.1977, P.G. Patwardhan & M.B. Nagarkar (**Holotype**, AMH 77.779).

Etymology: From the Latin *rupes*, rock, + *–cola*, dweller, a reference to the species habit on rock.

Thallus saxicolous, grey-brown colored. Ascocarps concolorous with the thallus, short, 0.2–0.5 mm long, simple, or irregularly branched, curved, immersed. Disc narrow, rarely ± open, pruinose. Exciple convergent, apically 1–2 striate, carbonized at the tips. Hymenium hyaline, laterally KI+ blue, clear, not inspersed. Asci 1–2-spored, hyaline, muriform, I+ blue violet, peripheral and central locules of more or less equal size, regularly arranged in rows, 88–147 × 25–42 µm.

Chemistry—Constictic, cryptostictic, and stictic (all major) and norstictic (minor) present (K+ yellow).

Ecology & distribution— collected from the tropical and sub-tropical regions of Meghalaya, Nagaland and Sikkim at ~1500–2000 m.

Additional specimens examined—**INDIA. Meghalaya:** Wiloe, 30.10.77, P.G. Patwardhan & M.B. Nagarkar, AMH 77.1092; Cherrapunjee Road, 27.10.77, P.G. Patwardhan & M.B. Nagarkar, AMH 77.816, 77.817; Mohmtheid-Cherrapunjee road, 28.10.77, P.G. Patwardhan & M.B. Nagarkar, AMH 77.1027, 77.1031, 77.1040; Mawrong, on Cherrapunjee Road, 27.10.77, P.G. Patwardhan & M.B. Nagarkar, AMH 77.2048; Mawsynram, 30.10.77, P.G. Patwardhan & M.B. Nagarkar, AMH 77.1135; Nagaland, 12 km to Zabuza, 7.11.77, P.G. Patwardhan & M.B. Nagarkar, AMH 77.1468; Sikkim, near Tangshi view point, Gangtok, 17.10.77, P.G. Patwardhan & M.B. Nagarkar, AMH 77.1947.

Remarks—*Diorygma rupicola* is morphologically very close to *D. hieroglyphicum* (Pers.) Staiger & Kalb, which differs in having a corticolous habit, divergent exciple, and ascospores with a gelatinous sheath. *Diorygma albocinerascens*, a corticolous species with similar exciple morphology and

spore size, can easily be distinguished from the new species in its much narrower (18–27 µm) ascospores and cryptostictic, methylstictic, norstictic and salazinic acids (all major).

Diorygma monophorum (Nyl.) Kalb et al., resembles *D. rupicola* in exciple characters, 1–2-spored asci, large and very broad ascospores (105–165 × 35–60 µm), but it differs in having hypostictic, hypoconstictic, and α-acetytylhypoconstictic acids (major or submajor).

Key to the species of *Diorygma* from India

1a. Ascospores exceeding 200 µm in length 2

1b. Ascospores not exceeding 200 µm in length 5

2a. Exciple distinctly divergent; disc exposed; thallus grayish white, powdery, smooth; ascocarps concolorous with the thallus, 1–3 mm long, emergent; disc yellowish to brownish; exciple divergent, non-carbonized; asci 1-spored; ascospores muriform, (135–)168–205 × 54–96 µm with 5–7.5 µm thick sheath; constictic (major) and stictic (major) acids present ..... ***D. rufosporum*** (Patw. & C.R. Kulk.) B.O. Sharma & Makhija

2b. Exciple distinctly convergent; disc hidden 3

3a. Norstictic acid absent; thallus corticolous, greenish white; ascocarps 0.5–9 mm long and 0.1–0.25 mm broad, simple, rarely branched, disc narrow, pruinose; exciple convergent, 2–6-striate, blackish brown at apices and dark brown laterally; asci 1-spored; ascospores muriform, 231–244 × 59–76 µm, consalazinic, constictic, cryptostictic, and stictic acids (major) present.... ***D. megaspermum*** Makhija et al.

3b. Norstictic acid present 4

4a. Thallus corticolous, glaucous, whitish to greenish gray; ascocarps 1–4 mm long simple to rarely branched, whitish, or concolorous with the thallus; disc slit like or broader, blackish brown to black, white pruinose when open; exciple poorly developed, 3–4 indistinct striation, blackish brown at apices, pale yellow to almost hyaline, convergent; asci 1–4-spored; ascospores muriform, 151–294 × 38–63(–84) µm, cryptostictic, norstictic, and stictic acids present ..... ***D. megistosporum*** Makhija et al.

4b. Thallus corticolous, smooth to cracked, pale glaucous green to olivaceous buff or whitish; ascocarps 1–6 mm long simple to rarely branched, immersed to semi-emergent, concolorous with the thallus; disc white, slit-like, later moderately open, white pruinose; exciple poorly developed, pale yellow, indistinctly 2–4-striate, blackish brown at apices; ascospores 1–2–4/ascus, 147–273 × 34–67 µm; constictic, cryptostictic, methylstictic, norstictic, and salazinic acids present ..... ***D. excipuloconvergentum***

5a. Ascospores exceeding 100 µm in length 6

5b. Ascospores not exceeding 100 µm in length 20

6a. Thallus saxicolous 7

6b. Thallus corticolous 8

7a. Thallus saxicolous, grey-brown colored; ascocarps concolorous with the thallus, short, 0.2–0.5 mm long, simple, or irregularly branched; exciple convergent, apically 1–2 striate, carbonized at the tips; asci 1–2-spored, muriform, 88–147 × 25–42 µm; constictic, cryptostictic, stictic (major), and norstictic (minor) present ..... ***D. rupicola***

7b. Thallus saxicolous, greenish to pale gray, rough; ascocarps concolorous, curved, short, round to elongate, simple to branched, very closely arranged, immersed to slightly emergent; disc narrow to broad; exciple divergent, non-carbonized, poorly developed, yellowish brown laterally and basally, 2–3 indistinct striate at the apical region; asci 1–6-spored; ascospores muriform, 143–172 × 29–34 µm; constictic, norstictic (trace) and stictic acids present ..... ***D. saxicola*** B.O. Sharma & Makhija

8a. Protocetraric acid present 9

8b. Protocetraric acid absent 10

9a. Thallus corticolous, whitish green; ascocarps concolorous with the thallus, circular, oval, short curved, 1–3 mm long; disc grayish, broad; exciple divergent, non-carbonized to carbonization sometimes restricted to the basal corners; asci 1-spored; ascospores muriform, 147–160 × 34–42 µm; protocetraric acid ..... ***D. pruinosum***

9b. Thallus corticolous, pale greenish-grey; ascocarps lirelline, concolorous with the thallus, 1–1.5 mm long, mostly simple, rarely branched; disc narrow, covered with white pruina; exciple convergent, non striate; asci 8-spored, muriform, 87.5–125 × 20–37.5 µm; cryptostictic, protocetraric and stictic acids present ..... ***D. agumbense***

10a. Asci 8-spored 11

10b. Asci more than 1-spored 12

11a. Thallus corticolous; exciple convergent; ascospores 1–8/ascus, 85–197 × 29–71 µm; constictic, stictic, and cryptostictic acids present..... ***D. megasporum***

11b. Thallus corticolous, ashy white; ascocarps white, straight to curved, branched, 0.5–2.5 mm long, immersed; disc 0.3–0.6 mm broad; exciple convergent to divergent, indistinctly striate; asci 1–8-spored; ascospores 75–145 × 24–33.6 µm; norstictic and stictic acids present ..... ***D. subalbatum*** (Patw. & Makhija) B.O. Sharma & Makhija

12a. Exciple distinctly convergent; disc hidden 13

12b. Exciple distinctly divergent; disc exposed 16

13a. Asci 1-spored 14

13b. Asci more than 1-spored 15

14a. Thallus corticolous, pale greenish-grey; ascocarps lirelline, concolorous with the thallus, 0.5–2 mm long; disc narrow, covered with white pruina; exciple convergent, non striate; asci 1-spored, muriform, 125–175 × 27.5–35 µm; norstictic and salazinic acids present..... ***D. dandeliense***

14b. Thallus corticolous, greenish grey with red tinge; ascocarps long flexuous, branched, curved, up to 9 mm long; disc slightly open, pale brown; exciple

convergent to divergent, non-carbonized; asci 1-spored; ascospores muriform, 105–113 × 33–42 µm; norstictic acid present ..... ***D. longilirellatum*** B.O. Sharma & Makhija

15a. Thallus corticolous, whitish gray, finely cracked; ascocarps 0.5–6 mm long simple, dendroidly branched, immersed, straight or curved, concolorous with the thallus; disc narrow, slit-like, light brown, slightly pruinose; exciple convergent, 2–3-striate, blackish brown at apices, asci 1–2-spored; ascospores 135–150 × 18–27 µm; cryptostictic, methylstictic, norstictic, and salazinic acids present ..... ***D. albocinerascens***

15b. Thallus corticolous, greenish-grey, dull; ascocarps concolorous with the thallus, 0.5–2 mm long, simple, curved, immersed; disc narrow, covered with white pruina; exciple convergent, non striate; asci 1–4-spored, muriform, 130–200 × 32.5–42.5 µm; norstictic and salazinic acids present ..... ***D. karnatakense***

16a. Norstictic and salazinic acids present 17

16b. Salazinic acid absent 18

17a. Thallus corticolous, glaucous grey, deeply cracked, distinctly verrucose; ascocarps crowded, concolorous with the thallus, irregular, short, 1–2 mm long; disc narrow to wide, sunken, 0.2–0.4 mm wide, reddish brown covered with white pruina; exciple, divergent, uncarbonized, base distinctly orange brown; asci 1-spored, muriform, 97–126 × 25–33 µm; norstictic and salazinic acids present ..... ***D. verrucirimosum***

17b. Thallus corticolous, white with brown tinge, smooth, thick; ascocarps rounded, lirellate, concolorous with the thallus, immersed, irregular, simple to branched, curved, 0.5–2.5 mm long; disc narrow to broad, 0.2–0.7 mm; exciple divergent, uncarbonized, laterally poorly developed, base distinctly orange brown; asci 1-spored, ascospores muriform, 105–147 × 33–37 µm; norstictic and salazinic acids ..... ***D. dealbatum***

18a. Peripheral spore locules distinctly smaller than the central ones. Thallus corticolous, rough; ascocarps 1–5 mm long, raised from the thallus; exciple non-carbonized, yellowish orange; asci 1-spored; ascospores muriform, 100–130 × 44–48 µm; norstictic and con-norstictic acids present ..... ***D. tuberculosa*** (Stirt.) Kalb et al.

18b. Peripheral and central spore locules of equal size 19

19a. Thallus corticolous, greenish with whitish tinge, smooth; ascocarps off white to concolorous with the thallus, immersed 1–4 mm long; disc broad, brown, 0.2–0.4 broad, pruinose; exciple non-carbonized, poorly developed, yellowish brown laterally and basally not striate; asci 1-spored; ascospores muriform, 80–131 × 42–52 µm, with a 5–15 µm thick sheath; stictic, constictic and norstictic acids present. ..... ***D. hieroglyphicum***

19b. Thallus corticolous, creamy white, grayish; ascocarps 0.4–4 mm long, round to irregular, branched, immersed to slightly raised; disc brownish grey covered by yellowish cream pruina; exciple divergent, non-carbonized, orange yellow at the

base; asci 1(–2)-spored; ascospores 84–134.4 × 29.4–42 µm, with a 2.3–5 µm sheath; constictic acid (trace), norstictic, and stictic acid (major) present. ..... ***D. junghuhnii*** (Mont. & Bosch) Kalb et al.

20a. Asci 1-spored 21

20b. Asci more than 1-spored 22

21a. Thallus corticolous, glaucous green with a yellow and or red tinge, uneven; ascocarps 1–6 mm long, simple to irregularly branched; disc brown, 0.2–0.3 mm broad; exciple non-striate, divergent, non carbonized, distinctly orange at the base; asci 1-spored; ascospores muriform, 79–96 × 29–33 µm; norstictic and salazinic acids present..... ***D. inaequale***

21b. Thallus corticolous, grayish white, smooth to rough, deeply cracked and appearing areolate; ascocarps concolorous with the thallus, 1–1.5 mm long, simple to branched, grouped, immersed; exciple hyaline, poorly developed, 2–3 striate, pale brown laterally, blackish brown at apices, convergent; asci 1-spored; ascospores muriform; 57–96 × 24–36 µm; consalazinic, constictic, cryptostictic, and stictic acids (major); Maharashtra state, [AMH 04.13] ... ***D. "patwardhanii"***

22a. Norstictic and salazinic acid present 23

22b. Salazinic acid absent..... 24

23a. Thallus corticolous, grayish, smooth to rough; ascocarps 1–2 mm long, simple, semi-emergent, concolorous with the thallus; exciple 2–3 striate, pale brown laterally, blackish brown at apices, convergent; asci 1–2-spored; ascospores muriform, 75–99 × 24–30 µm; norstictic, salazinic, and methylstictic acids present ..... ***D. panchganiense*** Makhija et al.

23b.Thallus corticolous, brownish white, ± smooth to rough; ascocarps concolorous with the thallus, 0.2–0.3 mm long mostly simple to rarely branched; disc brown, 0.4–0.5 mm broad; exciple 2–5 striate, apically dark brown, divergent; asci 2–4 spored; ascospores muriform, 76–92 × 21–25 µm; Norstictic and salazinic acids present ..... ***D. manipurense***

24a. Thallus corticolous, glaucous green, smooth to cracked; ascocarps 3–7 mm long simple to branched, immersed to emergent; disc reddish brown to blackish brown; exciple 2–5 striate, orange brown, carbonized at apices, convergent; asci 8-spored; ascospores muriform, 46–63 × 17–25 µm; stictic acid (major); Maharashtra state; [AMH 72.24] ..... ***D. "microsporum"***

24b. Thallus corticolous, greenish white, smooth to cracked; ascocarps 0.5–3 mm long simple to branched, concolorous with the thallus, fissure-like; exciple 2–3-striate, pale brown to blackish-brown at apices, convergent, asci 4–8-spored; ascospores muriform, 66–99 × 12–36 µm; constictic, cryptostictic, and stictic acids present ..... ***D. albovirescens*** Makhija et al.

(Note: species in quotations marks — *D. "patwardhanii"* and *D. "microsporum"* — are recorded by Makhija et al. (2009) and are probably new to science, but are not formally described due to scanty material.)

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Gyalidea minuta in Central Europe – new data on its distribution, ecology, and morphological variation

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Abstract — New localities of *Gyalidea minuta* in Central Europe are reported. The distribution, ecology, and morphological variation of the species are discussed and differentiating characters presented.

Key words — lichenized fungi, lichens, *Solorinellaceae*, Poland, Czech Republic

Introduction

The genus *Gyalidea* was resurrected by Vězda (1966) and by Vězda & Poelt (1991). Currently included in the *Solorinellaceae* (Baloch et al. 2010). *Gyalidea* was earlier referred to the *Asterothyriaceae* and *Gomphillaceae* (see Henssen & Lücking 2002, Lumbsch & Huhndorf 2010). The genus is characterized by a crustose thallus, zeorine apothecia with a mostly well-developed thalline margin, an *Ostropales*-type ascus, a non-amyloid hymenium with simple to sparsely branched and septate paraphyses, and hyaline, muriform (or transversally septate) ascospores (Vězda 1966; Vězda & Poelt 1991; Henssen & Lücking 2002; Aptroot & Lücking 2002). The thallus is usually inconspicuous and the apothecia small. *Gyalidea* is a cosmopolitan genus with over 40 species (Vězda & Poelt 1991; Kirk & Cooper 2009), many of which have been described in the last two decades (see Lumbsch et al. 2009). They are rather rare lichens, some known only from the type material or very few collections. Most species grow on soil, rocks, mosses, or plant debris, and only a few occur on tree bark. In Europe there are only two epiphytic species, *G. minuta* (van den Boom & Vězda 1995) and the recently described *G. fruticola* (Svensson & Thor 2007). Until recently, *G. minuta* had been found only in three localities in southwestern and

western Europe. Here we report the first record of *G. minuta* in Central Europe and discuss its distribution, ecology, and morphological variation.

Materials & methods

The specimens were morphologically examined by standard microscopic techniques. Hand-cut sections and squash preparations were examined in water, a 10% aqueous solution of KOH, and Lugol's solution. Measurements of well-developed free ascospores lying outside the asci were measured in water at ×1000 magnification. The description below is based on the isotype and newly collected specimens.

The taxon

Gyalidea minuta Van den Boom & Vězda, Mycotaxon 54: 423. 1995. FIG. 1

TYPE: Portugal, Algarve (holotype, herb. van den Boom 14875 [not seen]; isotype, herb. A. Vězda (PRA-V-05556!)).

THALLUS epiphloedal or partly endophloedal, corticolous, scattered among substrate wrinkles, grayish green, without visible prothallus. APOTHECIA sessile, hyaline with a brownish tinge, translucent when wet, 0.15–0.2 mm diam., 0.1 mm tall (0.2–0.4 × 0.1–0.15 mm in water preparation). HYMENIUM colorless, 45–75 µm tall. Paraphyses simple, not broadening towards the tips, 1.5–2.0 µm in diam., indistinctly septate. Asci cylindrical-clavate, wall slightly thickened at apex, 8-spored, 28–40 × 7–9 µm. Ascospores ellipsoid, with rounded to attenuate ends, muriform, with 3–5 transverse septa, and 1–3(–4) longitudinal septa, (9.5–)10–17(–20) × 4.5–7.5(–9) µm. PYCNIDIA not observed. CHEMISTRY not tested by TLC.

ECOLOGY – In western Europe the species has been reported from the bark of *Alnus glutinosa* and *Fraxinus* sp. trees in shady, humid forests and among shrubs (van den Boom & Vězda 1995, Sparrius et al. 2002).

In Poland, it has been found only in forest conditions. One locality is a narrow strip of land (ca. 50 m wide and about 2 km long) adjacent to a lake with an anthropogenic forest community of an unusual structure. The tree layer consists of middle-aged pine and the shrub layer of *Berberis* sp., *Crataegus* sp., *Euonymus europaeus*, *Rhamnus cathartica*, *Sambucus nigra* in which the common buckthorn (*R. cathartica*) dominates. The dense shrub-layer and the proximity to the lake give rise to highly humid sheltered conditions. *Gyalidea minuta* grows here on the bark of the buckthorns only and is intermixed with lichens common to this area (*Anisomeridium polypori* (Ellis & Everh.) M.E. Barr, *Bacidina sulphurella* (Samp.) M. Hauck & V. Wirth, *Coenogonium pineti* (Schrad. ex Ach.) Lücking & Lumbsch, *Lepraria incana* (L.) Ach., *L. lobificans* auct., *Melanelixia fuliginosa* (Fr. ex Duby) O. Blanco et al., *Micarea micrococca* (Körb.) Gams ex Coppins, *Psoroglaena abscondita* (Coppins & Vězda) Hafellner & Türk, *Parmelia sulcata* Taylor).

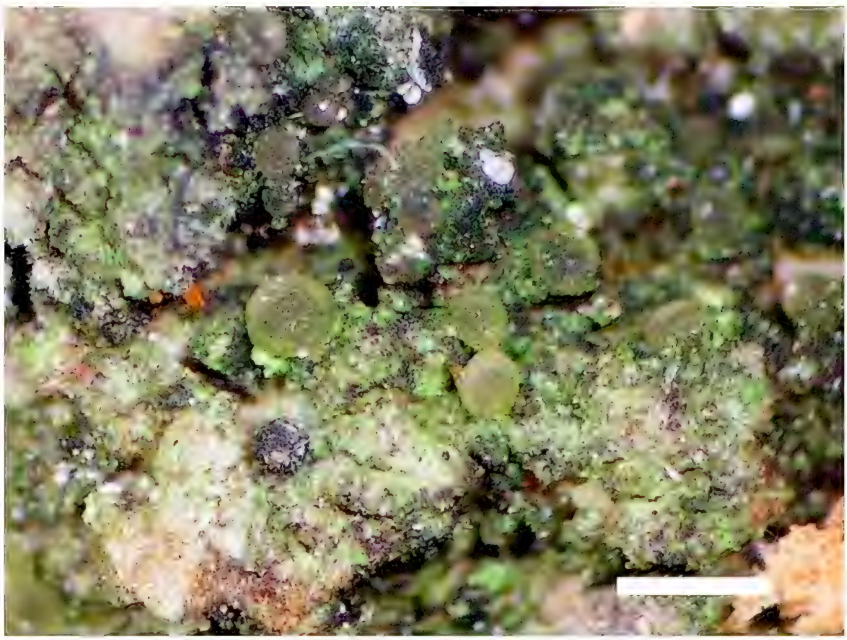

FIGURE 1. Habit of *Gyalidea minuta*. Scale bar = 0.5 mm.

The second locality is in a pine forest planted in soils that naturally favor oak–linden–hornbeam forest. In this habitat, *G. minuta* grows at the bottom of *Acer pseudoplatanus* trunks mixed with abundant *Bacidina sulphurella*. Other lichen species include *Lepraria incana*, *L. elobata* Tønsberg, and *Parmelia sulcata*.

In the Czech Republic *G. minuta* has been collected in a shady, damp forest of mostly *Alnus glutinosa* and *Fraxinus excelsior* within part of an old spruce–beech forest reserve. Here the species grows abundantly around bark rifts of *Fraxinus* trunks accompanied by *Lepraria* sp., *Lecanora pulicaris* (Pers.) Ach., and *Phlyctis argena* (Ach.) Flot.

GEOGRAPHICAL DISTRIBUTION (FIG. 2) –Known only from Europe, *G. minuta* had been previously reported from Portugal (van den Boom & Vězda 1995), northern France, and Belgium (Sparrius et al. 2002).

SPECIMENS EXAMINED: **POLAND**. **WARMIA–MASURIA**: OLSZTYN LAKE DISTRICT, Olsztyn, Łupsztych settlement, near lake Ukiel (53°46′50″N 20°24′20″E), 10.VII. & 10.XI.2001, leg. D. Kubiak (OLTC L–1425, PRA-V-03137, PRA-V-03138); 15.IX.2002, leg D. Kubiak (OLTC L–1976); 26.IV.2005, leg D. Kubiak (OLTC L–2879); 7.II.2009, leg. D. Kubiak (OLTC L–3113); Olsztyn, Dajtki settlement, near lake Ukiel (53°46′53″N 20°25′20″E): 6.VI.2009, leg. D. Kubiak (OLTC L–3190). **CZECH REPUBLIC**. **EASTERN**

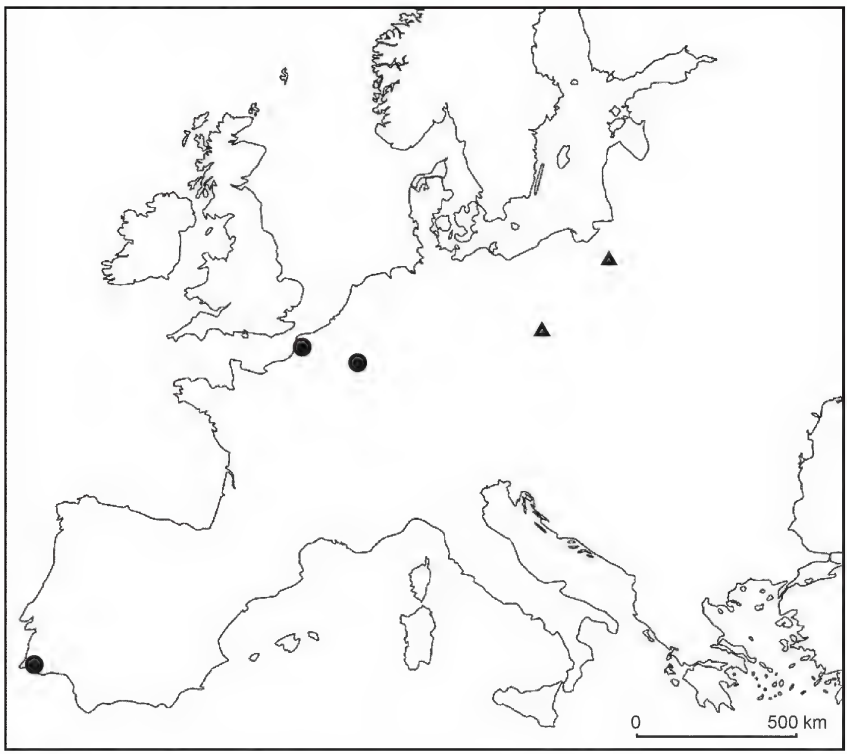

Figure 2. Distribution of *Gyalidea minuta*.
● – previously published localities, ▲ – new localities.

Bohemia: Železné hory Mts, Nasavrky–Horní Bradlo, Polom Nature Reserve (ca. 49°47′34″N 15°45′16″E), 24.IX.2009, leg. J. Malíček, J. Halda & A. Müller (herb. J. Malíček 2018).

Additional specimens examined: **PORTUGAL**: **Algarve**: Serra de Monchique, road 267 to S. Marcos da Serra (Alferce), 1.8 km E of crossing to Monchique (37°19.0′N 8°32.3′W), 28.VII.1993, leg. P.v.d. Boom (PRA-V-05556, isotype).

Comments – This constitutes the first report of *Gyalidea minuta* from Central Europe. The first specimen from Poland, found in 2001, differs from the type in apothecial shape and in the number and arrangement of septa. A. Vězda (pers. comm.) suggested that these differences were great enough to support a new species, but a final taxonomic decision was postponed in view of the small specimen size. No new material was found until 2009 when the first author found a larger population within 2 km of the first locality. In the same year the species was also found in the Czech Republic. Analysis of the rather rich material from both countries showed that all specimens represent a single

species, *Gyalidea minuta*. Detailed measurements have shown that the Central European specimens deviate slightly: the ascospores are slightly smaller with 3–4 transverse septa and the apothecia are generally larger and more flattened (Table 1). Furthermore, the exciples of dead apothecia are not persistent, as in the isotype specimen. These differences are probably due to the fact that the previously collected specimens are small and poorly developed and do not reflect the full variability of the species.

Table 1. *Gyalidea minuta* measurements: comparison between western and central European specimens.

	WESTERN EUROPEAN	CENTRAL EUROPEAN
Apothecia:		
Diam. (mm) in preparation	0.20–0.30	0.25–0.40
Hymenium:		
Height (µm)	50–60(–65)	45–75
Ascospores:		
Length (µm)	(9.5–)12–17(–20)	10–16
Width (µm)	5–9	4.5–7.5
Transverse septa	3–5	3–4
Longitudinal septa	1–3	0–3(–4)

It is worth noting that despite the relatively detailed and long-term observations in Poland, there are very few records of *G. minuta*. This may indicate that the species has an ephemeral nature, although it is also easily overlooked in the field.

Despite its variability, *Gyalidea minuta* is quite distinctive due to its occurrence on the bark of trees and shrubs, its very small, pale and almost translucent apothecia, and the barely visible thallus. The only other epiphytic *Gyalidea* species — the recently described *G. fruticola* (Svensson & Thor 2007), which also occurs on the bark of shrubs — differs in its larger, whitish grey to light yellowish brown apothecia and considerably longer, more septate ascospores. Additionally, *G. fruticola* has a well-developed true exciple that almost encloses the disc. In wet conditions, *G. minuta* resembles *Coenogonium pineti* in the field, but the microscopic features differ markedly. Rich photo documentation of the isotype and Central European collections is available on http://www.jjh.cz/foto/.

Acknowledgments

We are grateful to the late Antonín Vězda for his valuable taxonomic comments, Pieter P.G. van den Boom for the examination of our specimens, and Zdeněk Palice and Ludmila Kirschnerová for making the isotype specimen from PRA herbarium available. We are also grateful to Zdeněk Palice (Praha) and Harrie Sipman (Berlin) for very helpful comments and peer-reviewing the paper, and to Mark Leonard for critical reading and English correction.

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New species and record of *Sporidesmium* from southern China

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Abstract — Four species of *Sporidesmium* were collected from decaying twigs in tropical and subtropical forests in southern China. *Sporidesmium liquidambaris* sp. nov. on *Liquidambar formosana, S. antidesmatis* sp. nov. on *Antidesma ghaesembilla,* and *S. machili* sp. nov. on *Machilus chinensis,* are described, illustrated, and compared with closely related taxa. *Sporidesmium takashii* is recorded as new for the Chinese mycota.

Key words — conidial fungi, taxonomy

Introduction

The genus *Sporidesmium* (anamorphic *Pleosporales*) was established by Link (1809) with *S. atrum* Link as type species. Ellis (1958, 1971) defined the genus as having integrated, terminal, monoblastic, determinate or percurrent conidiogenous cells on distinctive, unbranched conidiophores, and acrogenous, solitary, transversely septate or distoseptate conidia. Under this generic concept, about 250 species are included in this genus. However, Sutton & Hodges (1979) and Hughes (1979) felt that *Sporidesmium* is heterogeneous, an opinion confirmed by Shenoy et al. (2006) who showed the polyphyletic nature of *Sporidesmium* and its allies. Kirk (1982) proposed the new genus *Sporidesmiella* P.M. Kirk for those species with cylindrical to cuneiform or obovoid, distoseptate conidia. Subramanian (1992) refined the generic concept of *Sporidesmium* in a stricter sense as 'conidiophores simple, determinate or irregular extending proliferations, conidia solitary, gangliar, acrogenous and euseptate', and proposed several novel anamorphic genera, including *Ellisembia* Subram., *Penzigomyces* Subram., *Repetophragma* Subram., and *Stanjehughesia* Subram., based on the absence/presence of conidiophores, type of conidiophore extension, and conidial septation (euseptate/distoseptate). Hernández-Guitiérrez & Sutton (1997) and Shoemaker & Hambleton (2001) further modified this approach by proposing *Imimyces* A. Hern. Gut. & B. Sutton, *Linkosia* A. Hern. Gut. &

B. Sutton, and *Imicles* Shoemaker & Hambl. However, Wu & Zhuang (2005) merged *Penzigomyces* into *Sporidesmium* and *Imicles* into *Ellisembia*, thereby expanding the generic concepts of *Sporidesmium* (euseptate) and *Ellisembia* (distoseptate) to include fungi with typically lageniform, doliiform, or nodose, percurrently extending conidiophores.

Sporidesmium is worldwide in distribution, usually found as a saprobe on rotten wood, dead branches, and decaying leaves of various plant species. (Ellis 1958, 1971, 1976, Wu & Zhuang 2005). During ongoing surveys of conidial fungi associated with woody debris in tropical and subtropical forests of southern China, four species clearly related to *Sporidesmium* sensu stricto were collected from decaying twigs. Three represent species new to science while the fourth is a new record from China.

Sporidesmium liquidambaris Jian Ma & X.G. Zhang, **sp. nov.** Fig. 1

MycoBank MB 563262

Conidiophora macronemata, nonramosa, 11–72 × 5.5–9.5 µm. Cellulae conidiogenae monoblasticae, integratae, terminales, per usque ad 7 extensiones lageniformes vel ampulliformes percurrentes. Conidia solitaria, obclavata, verruculosa, 11–26-euseptata, 110–165 × 20–24 µm, apicem versus ad 3–4.5 µm attenuata, basi truncata 4–5 µm lata.

Type: China, Fujian Province: Mount Wuyi, on decaying twigs of *Liquidambar formosana* Hance (*Hamamelidaceae*), 16 Aug 2009, J. Ma, (**Holotype** HSAUP H5059; **isotype** HMAS 146156).

Etymology: in reference to the host genus, *Liquidambar*.

Anamorphic fungi. Colonies on natural substrate effuse, brown to dark brown, hairy. Mycelium partly superficial, partly immersed in the substratum, composed of branched, septate, pale brown, smooth-walled hyphae, 1.5–3 µm thick. Conidiophores distinct, single or in groups, erect, unbranched, straight or flexuous, cylindrical, dark brown to black, smooth, septate, 11–72 × 5.5–9.5 µm. Conidiogenous cells monoblastic, integrated, terminal, brown to dark brown, smooth, 8.5–11.5 × 5–7 µm, with up to 7 lageniform or ampulliform percurrent extensions. Conidial secession schizolytic. Conidia holoblastic, acrogenous, solitary, straight or curved, obclavate, verruculose, brown to dark brown, apical cells pale brown or subhyaline, 11–26-euseptate, 110–165 µm long, 20–24 µm thick in the broadest part, tapering to 3–4.5 µm near the apex, 4–5 µm wide at the truncate base.

Comments –Among the known *Sporidesmium* species, *S. liquidambaris* resembles *S. ghanaense* M.B. Ellis (Ellis 1958), *S. matsutakashii* Subram. (Subramanian 1992), and *S. tengii* W.P. Wu (Wu & Zhuang 2005) in conidial shape. However, *S. liquidambaris* is distinguished from *S. ghanaense* (conidia 31–53 × 10–14 µm, 5–9-euseptate) and *S. tengii* (conidia 45–50 × 7–8.5 µm, 8-euseptate) by its larger conidia with more septa. It differs from *S. matsutakashii*

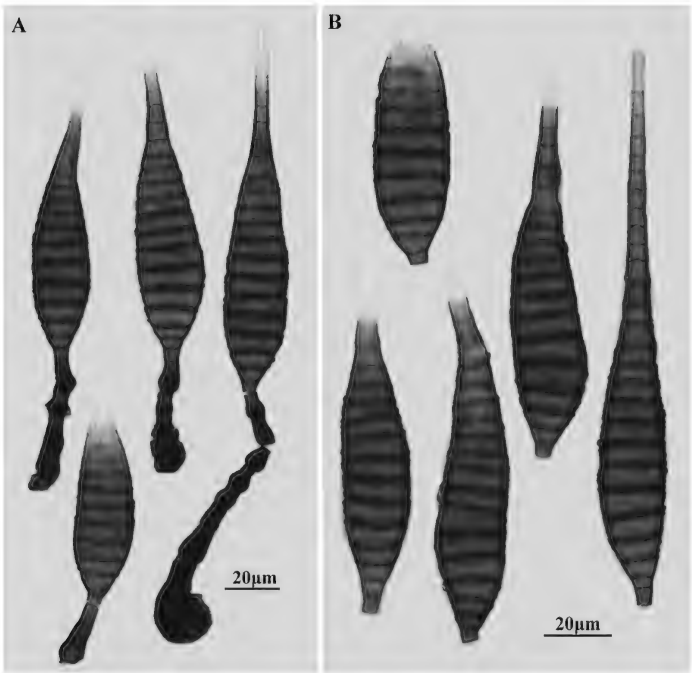

Fig. 1. *Sporidesmium liquidambaris*. A. Conidiophores with conidia. B. Conidia.

(conidia 9.5–14 μm wide) by its wider conidia that lack an apical mucilaginous appendage. In addition, the conidia of *S. liquidambaris* are verruculose while those of *S. ghanaense*, *S. matsutakashii,* and *S. tengii* are smooth.

Sporidesmium antidesmatis Jian Ma & X.G. Zhang, **sp. nov.** Fig. 2

MycoBank MB 563263

Conidiophora macronemata, nonramosa, 17–70 × 6–7.5 μm. Cellulae conidiogenae monoblasticae, integratae, terminales, determinatae, cylindrica. Conidia solitaria, obclavata, laevia, brunnea, 8–11-euseptata, 108–150 × 9–11 μm, apice rotundata, ad 3–4 μm lata, et muco subgloboso usque 12–24 μm diam. tecta, ad basim 4.5–6 μm lata.

Type: China, Hainan Province: Bawangling, on decaying twigs of *Antidesma ghaesembilla* Gaertn. (*Euphorbiaceae*), 11 Dec 2010, J. Ma, (**Holotype** HSAUP H5254; **isotype** HMAS 146157).

Etymology: in reference to the host genus, *Antidesma*.

Anamorphic fungi. Colonies on natural substrate effuse, brown to dark brown, hairy. Mycelium partly superficial, partly immersed in the substratum, composed of branched, septate, pale brown, smooth-walled hyphae, 1–2.5 μm

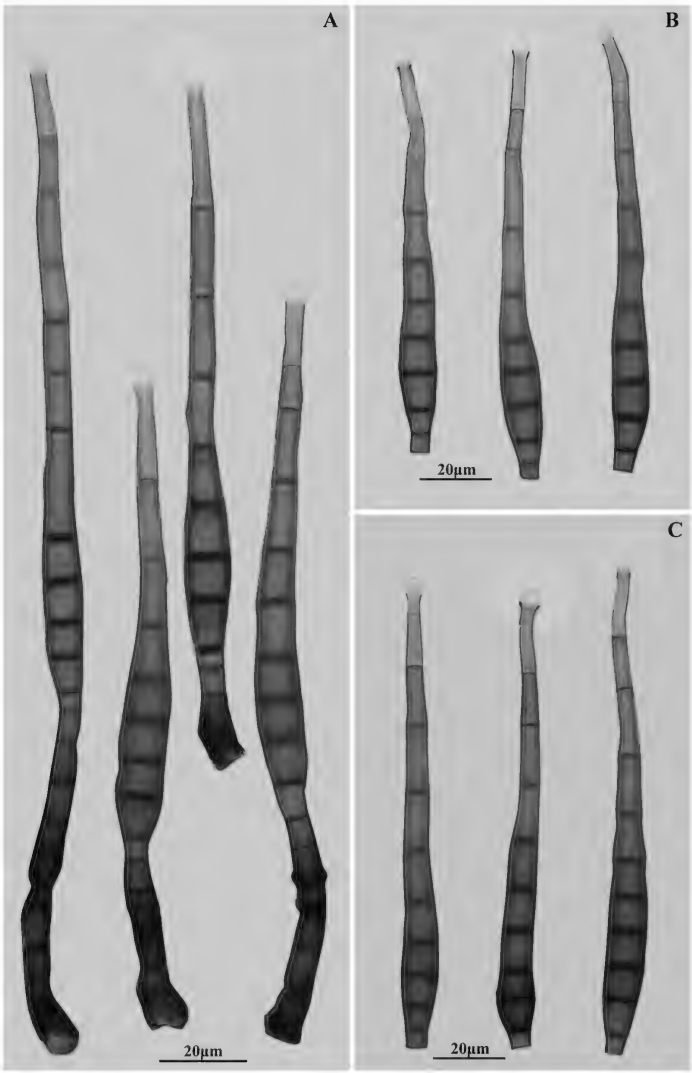

Fig. 2. *Sporidesmium antidesmatis.* A. Conidiophores with conidia. B–C. Conidia.

thick. Conidiophores macronematous, mononematous, single or in groups, erect, unbranched, straight or flexuous, cylindrical, brown to dark brown, smooth, septate, 17–70 × 6–7.5 μm. Conidiogenous cells monoblastic, integrated, terminal, determinate, cylindrical, brown, smooth, 4.5–7.5 × 5–6 μm. Conidial secession schizolytic. Conidia holoblastic, acrogenous, solitary,

straight or curved, obclavate, smooth-walled, brown, 8–11-euseptate, 108–150 µm long, 9–11 µm thick in the broadest part, tapering gradually towards the apex, base truncate, apex rounded, 3–4 µm wide, and invested in a subglobose drop of mucilage ca 12–24 µm diam, base 4.5–6 µm wide.

Comments – *Sporidesmium antidesmatis* superficially resembles *S. fragilissimum* (Berk. & M.A. Curtis) M.B. Ellis (Ellis 1958) and *S. eupatoriicola* M.B. Ellis (Ellis 1958). However, *S. antidesmatis* differs from *S. fragilissimum* (conidia 32–92 × 8–9 µm, verruculose) by its larger, smooth-walled conidia, and from *S. eupatoriicola* (conidia 60–195 µm long, apex 4–6 µm wide, 14–31-euseptate) by its slightly shorter conidia with narrower apices and fewer septa. In addition, the conidia of *S. antidesmatis* have an apical mucilaginous appendage while those of *S. fragilissimum* and *S. eupatoriicola* do not.

Sporidesmium machili Jian Ma & X.G. Zhang, **sp. nov.** Fig. 3

MycoBank MB 563264

Conidiophora macronemata, nonramosa, 15–60 × 3–5.5 µm. Cellulae conidiogenae monoblasticae, integratae, terminales, determinatae. Conidia solitaria, obclavata, ad longa rostrata, laevia, 5–7-euseptata, 70–160 × 5–7.5 µm, basi truncate, ad 1–1.5 µm lata, cellula apicali versus attenuate, pallide brunnea vel subhyalina, aseptata, rostro, ad usque 125 × 0.5–1.5 µm.

Type: China, Guangdong Province: Chebaling National Nature Reserve, on decaying twigs of *Machilus chinensis* (Benth.) Hemsl. (*Lauraceae*), 19 Oct 2010, J. Ma, (**Holotype** HSAUP H5407; **isotype** HMAS 146158).

Etymology: in reference to the host genus, *Machilus*.

Anamorphic fungi. Colonies on natural substrate effuse, brown, hairy. Mycelium partly superficial, but mostly immersed in the substratum, composed of branched, septate, pale brown, smooth-walled hyphae, 1.5–3 µm thick. Conidiophores macronematous, mononematous, single or in groups, erect, unbranched, straight or flexuous, cylindrical, brown, smooth, septate, 15–60 × 3–5.5 µm. Conidiogenous cells monoblastic, integrated, terminal, determinate, lageniform, brown, smooth, 13–23 × 3–4.5 µm. Conidial secession schizolytic. Conidia holoblastic, solitary, acrogenous, straight or curved, obclavate to long-rostrate, smooth-walled, brown to pale brown, 5–7-euseptate, 70–160 µm long (rostrum included), 5–7.5 µm thick in the broadest part, 1–1.5 µm wide at the truncate base, apex extended into a pale brown to subhyaline, aseptate, smooth rostrum, up to 125 µm long, 0.5–1.5 µm wide.

Comments – In terms of conidial morphology, *Sporidesmium machili* is similar to *S. circinophorum* Matsush. (Matsushima 1975), *S. takashii* (Subramanian 1992), *S. longirostratum* M.B. Ellis, and *S. tropicale* M.B. Ellis (Ellis 1958). However, *S. machili* differs from *S. circinophorum* (conidia 120–220 × 10–12 µm, 8–14-euseptate) and *S. tropicale* (conidia 80–250 × 12–15 µm,

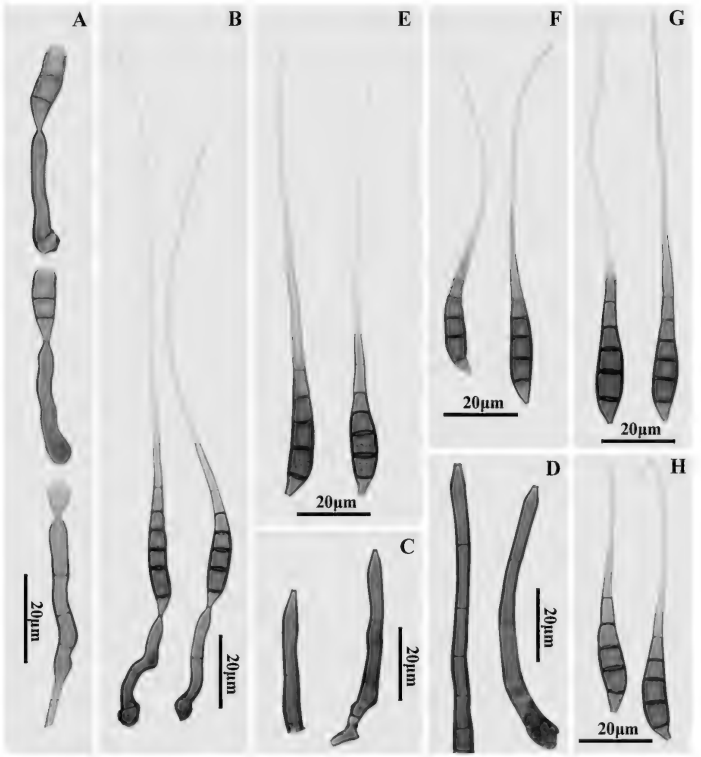

Fig. 3. *Sporidesmium machili*. A. Conidiophores with developing conidia. B. Conidiophores with mature conidia. C–D. Conidiophores. E–H. Conidia.

7–19-euseptate) by its smaller conidia with fewer septa, and from *S. longirostratum* (conidia 42–72 × 6–9 μm, 3–5-euseptate) by its longer and slightly narrower conidia with more septa. The conidia of *S. machili* are smooth-walled and are 5–7-eusepta while those of *S. takashii* are verruculose and 5–10-eusepta. In addition, the conidial rostrum in *S. machili* is filiform, pale brown to subhyaline and aseptate, which is obviously different from those of *S. circinophorum*, *S. takashii*, and *S. tropicale*.

Sporidesmium takashii Subram., Proc. Indian natn. Sci. Acad. B 58: 183, 1992. Fig. 4

Anamorphic fungus. Colonies on natural substrate effuse, brown, hairy. Mycelium partly superficial, partly immersed in the substratum, composed of branched, septate, pale brown, smooth-walled hyphae, 1–3 μm thick. Conidiophores macronematous, mononematous, single or in groups, erect, unbranched, straight or flexuous, cylindrical, brown, smooth, septate,

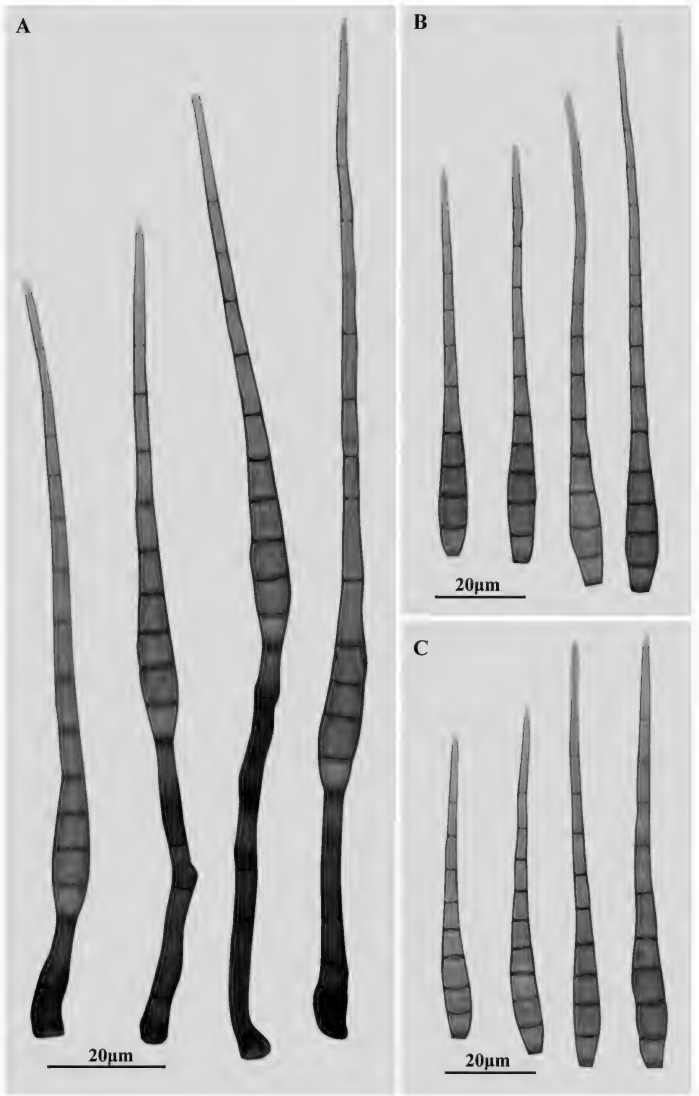

Fig. 4. *Sporidesmium takashii*. A. Conidiophores with conidia. B–C. Conidia.

13–60 × 3–5.5 μm. Conidiogenous cells monoblastic, integrated, terminal, determinate, cylindrical, brown, smooth, 10–16 × 3–4 μm. Conidial secession schizolytic. Conidia holoblastic, solitary, acrogenous, straight or curved, obclavate, rostrate, smooth-walled, brown to pale brown, 8–13(–16)-euseptate,

68–150 µm long, 5.5–7.5 µm thick in the broadest part, tapering to 1.5–2.2 µm near the apex, 2.5–4 µm wide at the truncate base.

Specimen examined: **China, Hainan Province**: Xinglong Tropical Botanical Garden, on decaying twigs of unidentified broad-leaved tree, 6 Dec 2009, J. Ma, HSAUP H5212, HMAS 146159.

Comments – Matsushima (1975) described this fungus as *Sporidesmium* sp. Subsequently, Subramanian (1992) assigned it as a new species, *S. takashii*, based on the earlier description. *Sporidesmium takashii* is morphologically most similar to *S. circinophorum* and *S. tropicale*. However, *S. takashii* conidia are distinctly smaller than those of *S. circinophorum* (conidia 120–220 × 10–12 µm) and *S. tropicale* (conidia 80–250 × 12–15 µm). In addition, *S. takashii* differs from *S. circinophorum* (conidia versicolored, apex 2.5–3.5 µm wide) and *S. tropicale* (conidia verruculose, apex 2–4 µm wide) in its concolorous, smooth-walled conidia with a narrower apex. Compared with the morphology of *S. takashii* described by Matsushima (1975) and Subramanian (1992), our collection fits well with the original description except for having more septa (8–16 vs 5–10) and smooth-walled conidia. This is the first report of this species in China.

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Phlebopus spongiosus sp. nov. (*Boletales, Boletinellaceae*) with a sponge-like tissue

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Abstract – A new species, *Phlebopus spongiosus*, is described with a peculiar sponge-like tissue in which the hollow spaces in the tubes are filled in by pleurocystidia-like elements, and a thin membrane of dissepiments encloses the immature pores. It occurs in citrus orchards (*Citrus maxima*) in southern Vietnam.

Key words – tropics, black bolete, edible mushroom, crypta

Introduction

Heim (1936) proposed *Phlebopus* as a subgenus of *Boletus* to accommodate *Boletus colossus* R. Heim. Singer (1936) elevated *Phlebopus* to genus with *P. colossus* (R. Heim) Singer as type species. Subsequently, Heinemann & Rammeloo (1982), Singer et al. (1983), and Singer (1986) emended the generic limits to encompass all taxa of *Phaeogyroporus* (Singer 1944). With 12 species (Kirk et al. 2008: 522), *Phlebopus* was classified in the *Boletinellaceae* (*Boletales*) by Binder & Bresinsky (2002).

Phlebopus is a tropical or subtropical genus with boletoid basidiomata producing an olivaceous brown spore print, ellipsoidal smooth basidiospores

(light microscope), and hyphae with clamp connections. In Southeast Asia, the two dominant edible taxa — *P. portentosus* (Berk. & Broome) Boedijn (Saccardo 1888, Boedijn 1951) and *P. marginatus* Watling & N.M. Greg. (Saccardo 1888, Watling & Gregory 1988) — are regarded as conspecific by Watling & Gregory (1988) and Watling & Li (1999).

During surveys of mycobiota in southern Vietnam, we collected in several orchards a taxon with a habit reminiscent of *P. portentosus*. In the present paper, this taxon is described and illustrated as new to science.

Materials & methods

Geographical coordinates of localities were determined by GPS (Garmin – eTrex Legend®) and Google Earth 5.0.11733.9347 software. Color notation in parentheses was determined using the Methuen Handbook of Colour (Kornerup & Wanscher 1981).

The microscopic characteristics were observed using a Nikon Labophot-2 equipped with DIC (Differential Interference Contrast) optics after hand-sectioning and mounting with 10% ammonium hydroxide aqueous solution. Abbreviations used: Q = mean length/width ratio measured from "n" number of spores; m = mean spore length and width; *P.* = *Phlebopus*.

A strain isolated from basidioma hyphae is maintained on modified Melin and Norkrans medium (Brundrett et al. 1996: 224), 20°C, in darkness.

All specimens are deposited in the herbarium of the Natural History Museum and Institute, Chiba, Japan (CBM).

Taxonomy

Phlebopus spongiosus Pham & Har. Takah., **sp. nov.** Pl. 1–6

Mycobank MB 518549

Differs from other *Phlebopus* species by sponge-like tissues with cystidioid elements filling hollow spaces in immature tubes, a thin membrane of subcylindrical dissepiments enclosing the immature pores, and a dark brown spore print.

Type: Vietnam. TienGiang: MyTho City, Highway 1A (UTM, 0646165, 1151001), 20 Jan 2006, N.D.H Pham (**Holotype**, CBM FB-38014)

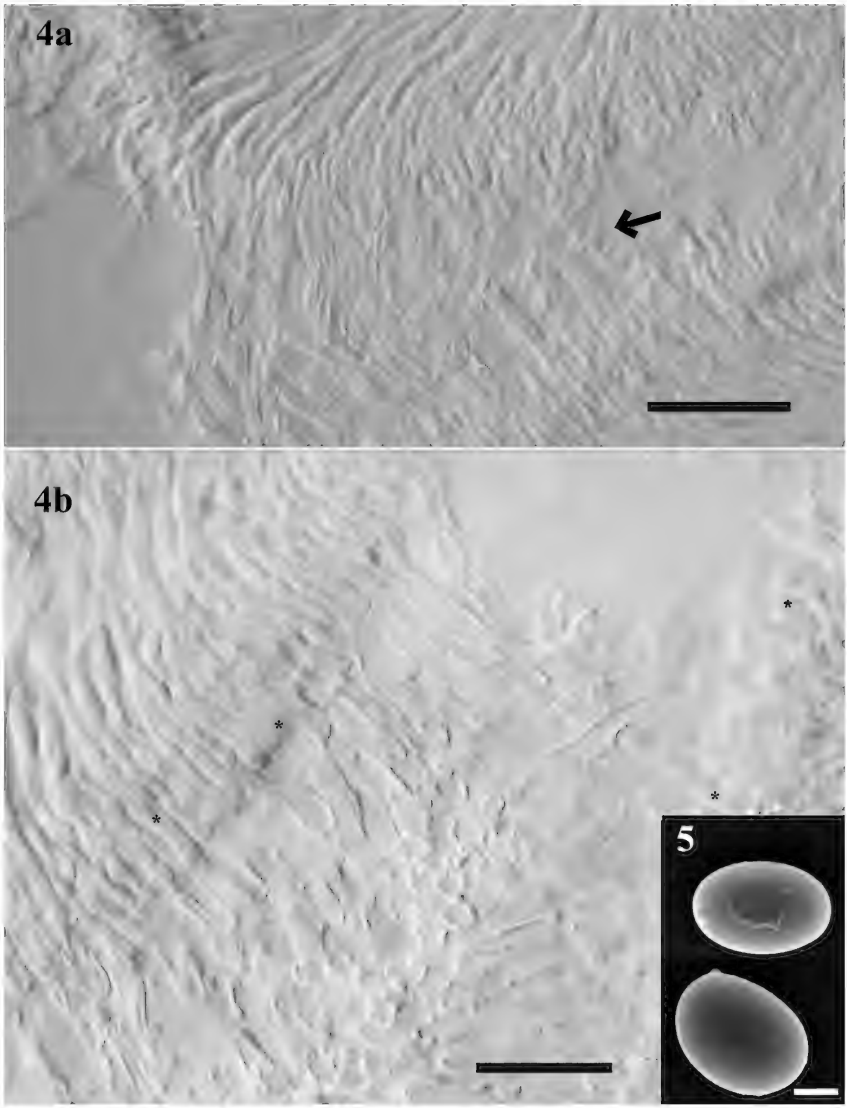
Etymology: From the Latin, referring to the sponge-like tissue

Macrocharacters – Pileus 50–120(–500) mm broad, at first hemispherical with incurved margin, then becoming plano-convex to convex with decurved margin; surface dry, at first subtomentose, then glabrescent; brown to yellowish brown (5D7-8 to 5E7-8) overall, sometimes tinged with olive (3F4-5) on old specimens. Flesh 10–20 mm thick at the center of pileus, soft, yellow (3A7 to 3B7-8) to vivid yellow (3A8), in places gradually bluing when exposed; odor indistinct when fresh; taste after cooking slightly like persimmon rind. Hymenophore decurrent to subdecurrent at first, then more or less depressed around the stipe; tubes ≤ 3 mm long in dried material, vertically arranged; hymenium usually with two thirds of the tubes filled in with yellowish orange

FIGURES 1–3. *Phlebopus spongiosus*. 1: Basidiomata in its habitat. 2: Vertical section of the immature basidioma; bar = 2 cm. 3: Vertical section of the hymenophore in dried specimens showing the sponge-like tissue arrow); bar = 1 mm.

(5A7-8 to 5B7-8) to yellowish brown (5E5-6 to 5F5-6), sponge-like tissue projecting from the sides of the tube walls, but this occluding tissue disappearing in old specimens; pores small (1–3 per mm), angular to subcircular, more elongated around the stipe, covered by a thin membrane in young specimens, brown (7D8-7E8) when young, then brown (7E5-6) to dark brown (7F5-6). Stipe 80–150 × 15–50 mm, central, subequal or somewhat enlarged at the base, sometimes tapering downward, solid; surface dry, subtomentose to tomentose, longitudinally striate toward the base; entirely yellowish brown (5E4-8 to 5F4-8), becoming darker where handled; basal mycelium brownish yellow. Spore print dark brown to reddish brown (8F5-6).

MICROCHARACTERS – Basidiospores m = 8.3 × 6.4 µm [7.9–8.8(–9.1) × 5.9–7.1(–7.4) µm, n = 47, Q = 1.30], ovoid to shortly ellipsoid, smooth under the light microscope, brown (5D6-7), inamyloid, thick-walled. Basidia 21–27 × 8–10 µm (without sterigmata), clavate, 4-spored; sterigmata 3–4 µm long. Hymenophoral trama divergent-bilateral of the *Boletus* subtype, hyaline, with gelatinized hyphae 4.5–10 µm diam.; mediostratum 80–110 µm diam. when young, 40–60 µm diam. at maturity. Sponge-like tissue composed of heteromorphous terminal cells occasionally protruding into the tube lacunae from the sides of the tube walls; constituent elements numerous, (29–)45–53 × 4.5–7.4 µm, cystidioid but not truly pleurocystidia, broadly clavate or fusoid-ventricose to ventricose-rostrate, thin-walled, with yellow (4B-5 to 4A-6)



Figures 4–5. *Phlebopus spongiosus*. 4: Vertical section through part of the hymenophore showing the sponge-like tissue (arrow) made up of heteromorphous terminal cells (*) unusually protruding into the lacunae of the tubes from the sides of the tube walls; bar = 50 µm in a, = 30 µm in b. 5: SEM figures of basidiospores; bar = 2.5 µm.

intracellular pigment, converging toward the center of the tube chamber at least in young specimens but gradually disrupted. Dissepiments resembling

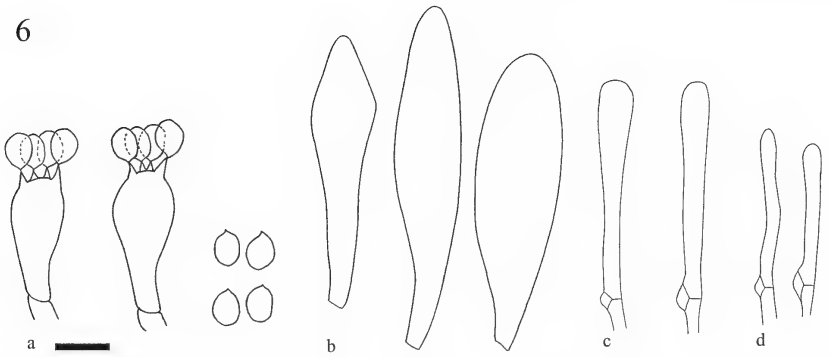


FIGURE 6. *Phlebopus spongiosus*. A: Basidiospores and basidia. B: Heteromorphous terminal cells protruding into the lacunae of the tubes from the sides of the tube walls. C: Dissepiments. D: Terminal cells of the pileipellis. Bar = 10 μm.

cheilocystidia, remarkably projecting beyond the hymenium, 25–33(–42) × 4–5(–6.5) μm, subcylindrical to cylindrical-clavate, thin-walled, distinctly clamped at base, with yellow (4B-5 to 4A-6) intracellular pigment, forming the membranous thin layer on the immature pore surface but gradually collapsing. Pileipellis consisting of repent, appressed, interwoven hyphal elements; terminal cells 22–29 × 3.3–4.7 μm, cylindrical, thin-walled, with yellow (4B7-8) intracellular pigment, golden yellow to brownish (5B7 or 5C7) in the wall mass. Pileitrama composed of cylindrical, loosely interwoven hyphae 4-6 μm diam., colorless or greyish yellow, smooth, inamyloid, thin-walled. Stipitipellis densely constructed, brown (6D8 or 6E7); terminal cells similar to those of the pileipellis. Stipe trama composed of longitudinally arranged, cylindrical hyphae 2-4.5 μm diam., thin-walled, colorless or brownish (6C4-6C5), inamyloid, rather compactly arranged compared to the pileitrama. Clamp connections constantly present in all tissues and in vitro.

HABITAT – Almost year-round, solitary to scattered in citrus farms, (dominated by *Citrus maxima* (Burm.) Merr).

ADDITIONAL SPECIMENS EXAMINED: **VIETNAM. TIENGIANG**: MYTHO CITY, Highway 1A (UTM, 0646165, 1151001), 25 Jun 2007, N.D.H Pham (CBM FB-38670; culture Phle01, Laboratory of Microbiology, Biotechnology Center of HoChiMinh City). **HOCHIMINH CITY**: TANPHU DIST., Cautre Company, 16 May 2007, N.D.H Pham (CBM FB-38017); DIST. 12, Biotechnology Center of HoChiMinh City (10°50′58.95″N, 106°37′03.89″E), 30 Mar 2008, N.D.H Pham (CBM FB-38671).

Discussion

The most distinctive features of *Phlebopus spongiosus* are the sponge-like tissue composed of numerous fusoid-ventricose to ventricose-rostrate pleurocystidioid elements, the subcylindrical to subclavate dissepiments

forming a thin membrane that covers the immature pores, and the dark-brown spore print not found in any other known *Phlebopus* species.

Although its dark brown spore print appears foreign to *Phlebopus* sensu Singer (1986), the shortly ellipsoid smooth basidiospores and clamp connections merit placing *P. spongiosus* within *Phlebopus* (Singer 1986).

Its medium to large dark yellowish-brown boletoid basidioma bears a superficial resemblance to the Southeast Asian *P. portentosus* (Boedijn 1951, Heinemann & Rammeloo 1982, Singer et al. 1983) and *P. marginatus* originally described from Australia (Watling & Gregory 1988, Grgurinovic 1997). Both taxa differ from *P. spongiosus* in their olivaceous brown spore print and lack of distinct hymenial cystidia (Boedijn 1951; Pegler 1986; Watling & Li 1999).

It should be noted that *P. spongiosus* basidiomata are sometimes accompanied by a crust-like mycelial structure around nearby citrus tree roots reminiscent of "crypta" as defined by Singer ("sleeve-like formations around tree roots in tropical and subtropical conditions;" 1986: 10). Further critical investigations, including cultural and molecular studies, are needed to confirm its relationship with *P. spongiosus* basidiomata.

The crypta-like mycelium of *P. spongiosus* is comparable to that of three neotropical taxa: *P. tropicus* (Rick) Heinem. & Rammeloo (Singer 1944, Singer & Digilio 1957, Heinemann & Rammeloo 1982, Singer et al. 1983), *P. beniensis* (Singer & Digilio) Heinem. & Rammeloo (Heinemann & Rammeloo 1982, Singer et al. 1983), and *P. brasiliensis* Singer (Singer et al. 1983). Those taxa differ from *P. spongiosus* mainly by lacking distinct hymenial cystidia and having olivaceous-brown spore print. Moreover, according to the *Phlebopus* key by Singer et al. (1983), the wide pores (≥1 mm diam.) or boletinoid/subgyrose hymenophore separate *P. tropicus* from *P. spongiosus*. Miller et al. (2000) noted that Puerto Rican *P. beniensis* specimens had well-developed cheilocystidia but provided no information regarding the presence or absence of pleurocystidia.

Phlebopus spongiosus also shares well developed fusoid-ventricose to ventricose-rostrate pleurocystidia-like elements with *P. cystidiosus* Heinem & Rammeloo from Ethiopia (Heinemann & Rammeloo 1982). However, the lack of clamp connections causes *P. cystidiosus* to seem out of place in *Phlebopus*. Its glabrous non-reticulate stipe, ellipsoid basidiospores, and absence of clamp connections make *P. cystidiosus* seem more closely related to *Rubinoboletus* Pilát & Dermek (Heinemann & Rammeloo 1983, Watling & Gregory 1988, Li & Watling 1999, Watling & Li 1999).

The thin membrane enclosing the immature pores might also suggest a relationship between *P. spongiosus* and the secotioid boletes (Thiers 1984). However, the secotioid boletes are generally associated with an often misshapen pileus, irregularly oriented tubes, and a typically less-developed stipe, characters not seen in *P. spongiosus*.

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Type studies in *Helvella* (*Pezizales*) 1

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Abstract — Sixteen type and some additional specimens representing fifteen species assigned to *Helvella* were studied with the aim of providing new data for their improved delimitation and recognition. Specimens were received on loan from DAOM, FH, H, K, MA, MICH, S and UPS. A synoptic key to the studied species is provided to contribute to a better understanding of the genus and the taxonomy of the morphospecies. Original descriptions are emended to include data on the anatomy of the apothecial excipulum and stipe layers, apothecium and stipe reactivity to Melzer's reagent, and ascospore ornamentation. Reasons are given for maintaining *H. subglabra, H. atra, H. griseoalba,* and *H. costifera* as independent taxa and for considering *Helvella hyperborea* a synonym of *H. costifera*. Previously imprecise descriptive terms are clarified.

Key words — *Ascomycota*, holotype, isotype, lectotype, revision

Introduction

The genus *Helvella* L. includes approximately 52 species (Kirk et al. 2008). Its distribution is basically north temperate, with only one tropical species recognized (Dissing 1979). Traditional taxonomy of the genus has been based mainly on ascoma shape and color and the presence or absence of projecting hyphae on the sterile surface (ectal excipulum) of the apothecium (Dissing 1966b, Weber 1972, Abbott & Currah 1997). Characters such as ascus development (aporhynchous or pleurorhynchous), ascospore shape and size, paraphysis color, and the presence of pigment in the apothecium ectal excipulum cells have proven to be of taxonomic value in some species (Weber 1972; Harmaja 1977b, 1979; Abbott & Currah 1997).

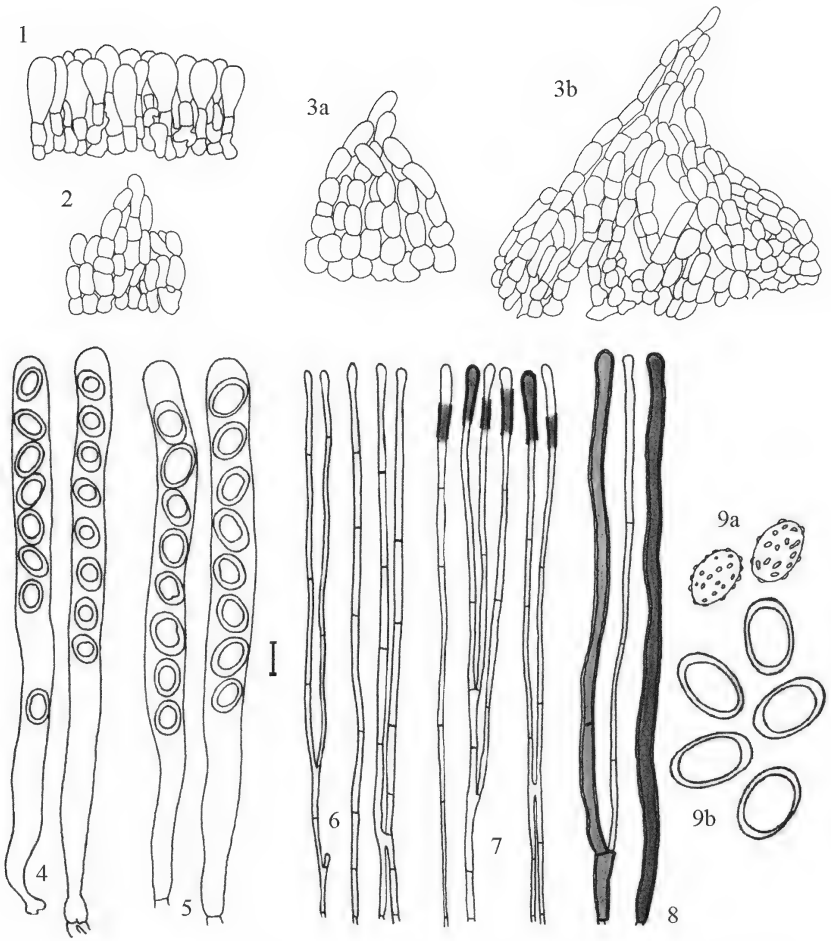
Although Nannfeldt (1937) proposed using the term "apothecium" specifically for *Helvella* to describe the entire ascoma, Weber (1972, 1975) and

Abbott & Currah (1988, 1997) used "apothecium" exclusively for the upper ascoma — i.e., the hymenium and its immediate supporting tissues — without the stipe. Eckblad (1968) commented that some authors carefully avoid the term apothecium for pileate forms like *Helvella*, whereas Dissing (1966b) and Calonge & Arroyo (1990) employed this term loosely, either for the complete ascoma or just for the upper part. Here, we follow Weber's (1972) concept, applying the term apothecium only for the upper ascoma, except that where she differentiated stipe tissues with "ectal excipulum" and "medullary excipulum," we use "outer layer" and "inner layer," because in discomycetes "excipulum" currently is interpreted as the "tissue or tissues containing the hymenium in an apothecium" (Kirk et al. 2008).

In all *Helvella* species the medullary excipulum and stipe inner layer are composed of "textura intricata" (interwoven hyphae) while the ectal excipulum and stipe outer layer are a "textura angularis" (almost isodiametric cells without intracellular spaces) (Korf 1952, Dissing 1966, Eckblad 1968, Weber 1972). These cells are arranged in rows perpendicular to the surface. In glabrous ascomata, the ectal excipulum external layer is a palisade of clavate hyphal end-cells, ending more or less at the same level (Fig. 1) (Weber 1972). Hairy ascomata have an ectal excipulum outer layer that Eckblad (1968) described as "consisting of more or less swollen chains of cells often converging into distinct clusters." These clusters are called "hyphal fascicles" (Weber 1972) or "chains of cells and/or fascicled hyphal chains" (Abbott & Currah 1997). Macroscopically, they give the appearance of pubescence or hairs of different lengths (Figs 2–3) and are important in the taxonomy of the genus (Dissing 1966). Descriptive terms used in the literature — "finely pubescent," "furfuraceous," "granulose," "hairy," "pruinose," "pubescent," "roughened," "velvety," "villose" (Dissing 1966; Weber 1972, 1975; Dennis 1981; Abbott & Currah 1997) — have generated confusion through their different meanings, incorrect application, and/or the lack of consistent usage. Kaushal (1991) alone consistently uses only "pubescent" to describe the hairs; furthermore he included the hyphal fascicle length for each species.

Only three papers have described the stipe tissue: Gómez & Herrera (1965) and Weber (1972, 1975). Gómez & Herrera concluded that because stipe tissue is continuous with apothecium tissue, it is not a useful character. Although Weber (1972) described both tissues as similar in color and structure, she did indicate color differences between tissues in some species descriptions.

Pigment distribution, mainly in ectal excipulum cells (see Figs 19–21) and paraphyses — i.e., intracellular (cytoplasmic), in the cell wall, or encrusting (deposited pigment forming an irregular crust on the wall) — is controversial. Eckblad (1968), Weber (1972), and Harmaja (1977b, 1979) considered pigmentation to be diagnostic. Eckblad (1968) wrote it is a characteristic mostly



Figs 1–9. 1: Ectal excipulum of a glabrous apothecium (*Helvella leucopus* var. *populina*). 2: Hyphal fascicle of the outer layer of a subpubescent stipe (*H. griseoalba*). 3: Hyphal fascicle of the ectal excipulum of a pubescent apothecium (*H. costifera*)—a: short, b: large. 4: Pleurorhynchous asci (*H. pocillum*). 5: Aporhynchous asci (*H. crassitunicata*); 6: Common paraphyses (*H. subglabra*). 7: Paraphyses with a thick-walled brown cap or collar at the apex (*H. paraphysitorquata*). 8: Thick-walled paraphyses (*H. crassitunicata*). 9: Ascospores (*H. solitaria*) —a: ornamented, b: smooth. Scale bars: 1–8 = 20 µm, 9 = 8 µm.

used on species level in *Pezizales*, but "[u]nfortunately too many descriptions simply state whether the paraphyses are coloured or not, not where the pigments are located." With respect to *Helvella*, Eckblad (1968) noted, "[c]hemically and genetically it is probably a very short step from pigmented paraphyses to

hyaline ones, e.g. from the greyish-black paraphyses of *Helvella lacunosa* to the colourless paraphyses of *H. crispa*." Weber (1972) recognized two patterns of pigment distribution: 1) intracellular pigments, occurring in species with buff, tan or brown ascomata, and 2) pigments associated with hyphal walls, characteristic of gray to black species. On the other hand, Dissing (1966b) and Abbott & Currah (1997) considered pigmentation as highly variable and of limited use in circumscribing *Helvella* species. Dissing (1966b) indicated it is difficult to assess, especially in species where hymenium color variation is very large (as in *H. lacunosa* Afzel.) and hymenium color ranges from pale gray to gray brown to black due to pigments in the paraphyses. Only Dissing (1964, 1966b) went beyond just describing the colors of the structures; he also evaluated the color before and after treatment with cotton blue. Unfortunately, no further works analyze this issue.

Chemical reactions to Melzer's reagent have not been considered as diagnostic characters for structures other than ascospores or asci. Although excipulum cells in other ascomycete genera (*Perrotia*, *Hymenoscyphus*, *Epibryon*) show a hemiamyloid reaction, its taxonomic value is uncertain (Baral 1987).

Helvella ascospores have limited taxonomic value. In fact, their size is a key feature only for *H. pocillum* and *H. crassitunicata* (Weber 1975; Harmaja 1976), while the fusoid to subfusoid shape (differing from the typically ellipsoid ascospores found elsewhere) is found only in *H. macropus* (Pers.) P. Karst. (Abbott & Currah 1997) and *H. terrestris* (Velen.) Landvik (Landvik et al. 1999). Ascospore ornamentation is highly complex and a subject of controversy. Dissing (1964) and Dissing & Nannfeldt (1966) regarded ornamented ascospores in *Helvella* as immature, while Weber felt (1972) that they corresponded to the mature condition. Eckblad (1968) described ascospores with "false ornamentation," and Schumacher (pers. com.) mentioned that during the final stage of ascospore development, some secondary wall remnants might adhere to the ascospore surface, producing a highly inconsistent pattern; typically, a number of ascospores do not get such adherences at all and consequently remain smooth. On the other hand, Abbott & Currah (1997) gave to this character a high value, because they considered verruculose ascospores unique to the subgenus *Macropodes* (Dissing) S.P. Abbott.

Species concepts are similarly varied. Some authors with a narrow species concept, such as Harmaja (1976, 1977a,b, 1979), segregated species based on subtle differences. Others with a wider species concept (e.g., Abbott & Currah 1997) use fewer diagnostic characters to separate *Helvella* species. The purpose of our study was to review selected *Helvella* type specimens to delimit morphospecies and to determine which characters could be used in taxonomic and phylogenetic studies of the genus. We present five sets of types belonging to species with problematic delimitations: 1) *H. costifera* vs. *H. hyperborea*

and *H. griseoalba*; 2) *H. crassitunicata* vs. *H. pocillum*; 3) *H. subglabra* vs. *H. atra*; 4) *H. solitaria* vs. *H. queletii* and *H. ulvinenii*; and 5) *H. verruculosa* vs. *H. dryadophila*. We also add to the earlier descriptions of *H. leucopus* var. *populina*, *H. maculata*, *H. paraphysitorquata*, and *H. robusta*. Finally, we suggest a set of standard terms to clarify descriptions of *Helvella* species.

Materials & methods

Sixteen type as well as some additional specimens from eight herbaria (DAOM, FH, H, MA, MICH, O, S, UPS) were studied. Herbarium abbreviations follow Holmgren et al. (1990). Free-hand sections from apothecium and stipe were made with a razor blade directly from dry specimens. Sections and mycelium were placed first in 70% ethyl alcohol and subsequently in water to rehydrate the tissues. Specimens were examined under a Zeiss Axioskop 2 plus microscope with a drawing tube. Microphotographs were taken under a Zeiss Axioskop 40 microscope using Axio Vision 4 software. Ascospore measurements were made on mature free spores outside the asci, or on those attached to the hymenial surface, stipe, or basal mycelia. Mounts from apothecium, stipe, and mycelium were also made in Melzer's reagent (without pre-treatment in KOH) and cotton blue. We follow in part the terminology used by Baral (1987) to describe the hemiamyloid reaction in Melzer's reagent ('Melzer's'): "rr+" for hemiamyloid ("solely red") reaction and "rr–" for negative reaction. All measurements and features recorded are from type specimens. Where additional non-type specimens were studied for some species, observations that differ from those of the type are indicated under "Remarks" but not included in the technical description.

Terms used to refer to the surface of the apothecial ectal excipulum and stipe outer layer are: GLABROUS (FIG. 1), SUB-PUBESCENT (for fascicled hyphal chains < 50 µm long; FIG. 2), and PUBESCENT (for fascicled hyphal chains > 50 µm long; FIG. 3). As the hairs' length can vary in the last case, their measurements are given in the descriptions. See also "Results & discussion".

Results & discussion

We studied sixteen type specimens and present only new information or data that differs from the protologue or previous publications. In addition to the characters established by previous authors (Dissing 1966b; Calonge & Arroyo 1990; Abbott & Currah 1997), we found that pigmentation of apothecial cells, stipe tissue anatomy, and the reddish (hemiamyloid) reaction in Melzer's reagent (FIGS 10–11) are important characters that contribute to species recognition in *Helvella*. Because we found no variation in the basal mycelium of the stipe, which is universally composed of smooth-walled, hyaline, rr–, interwoven hyphae, we do not include it in the species descriptions. Nor do we make further mention of the fact that ascospores of all species have both cyanophilic cytoplasm and ornamentation (when present).

Regarding the value of pigmentation in the structures, we think it is important to study it from two perspectives: a) location and b) intensity. Concerning

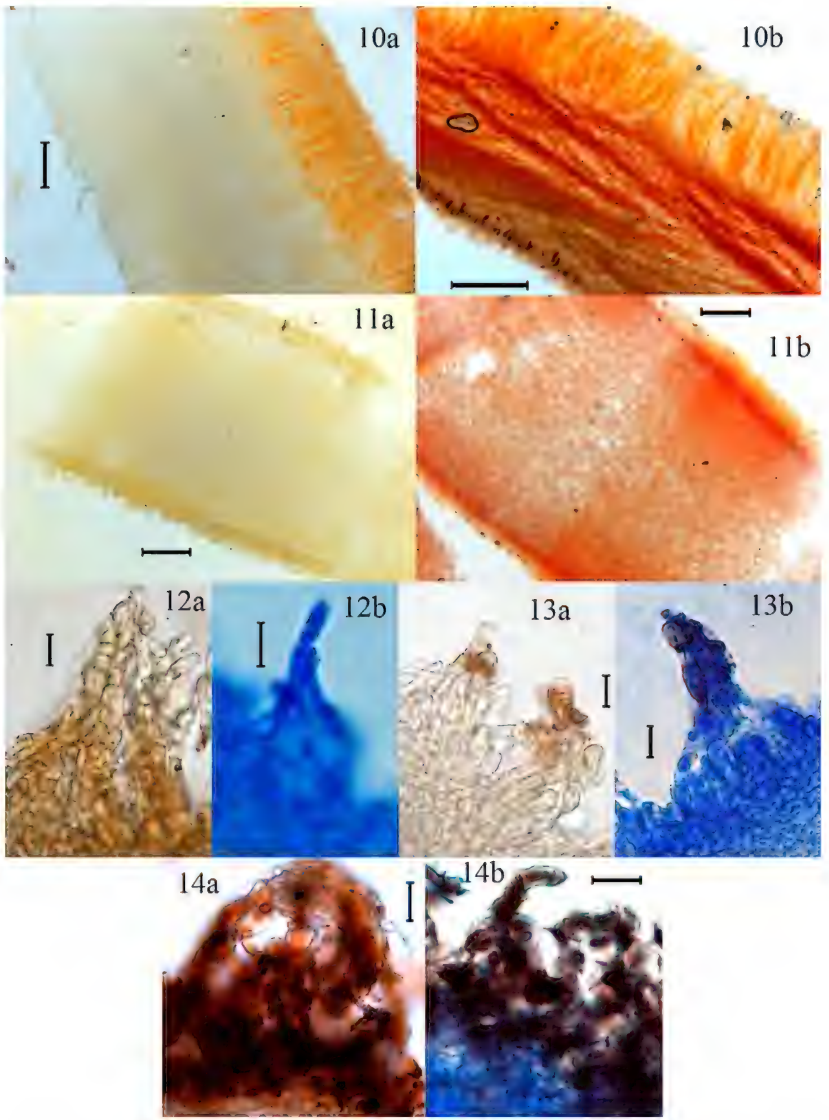
location, pigment may occur in the cytoplasm, in the wall, or encrusted on the wall (Figs 19–21). We observed that while the first two are constant within species, encrusted pigments are variable. For example, in *H. costifera* there are specimens with encrusted paraphyses and others without such incrustations. On the matter of pigment intensity, we return to cotton blue used first by Dissing (1964) to note that there are species where the cytoplasmic pigment color is so intense that it is still visible even after adding cotton blue, while others have lighter pigment no longer visible when adding the colorant (Figs 12–17). We realized that this characteristic is constant among specimens of the same species. This also happens with the pigment in the cell wall.

As indicated in the introduction, only Weber (1972, 1975) described the stipe tissue. We consider this character taxonomically valuable for the following reasons: 1) the hyphal fascicle lengths among specimens of the same species are more constant than in the apothecial sterile surface (see *H. hyperborea* remarks); 2) the pigments in the ectal excipulum and stipe outer layer can differ in water and cotton blue (see *H. ulvinenii* remarks; Fig. 22); and 3) the hemiamyloid reaction can occur in stipe tissue but not in the excipulum (e.g. *H. leucopus* var. *populina*).

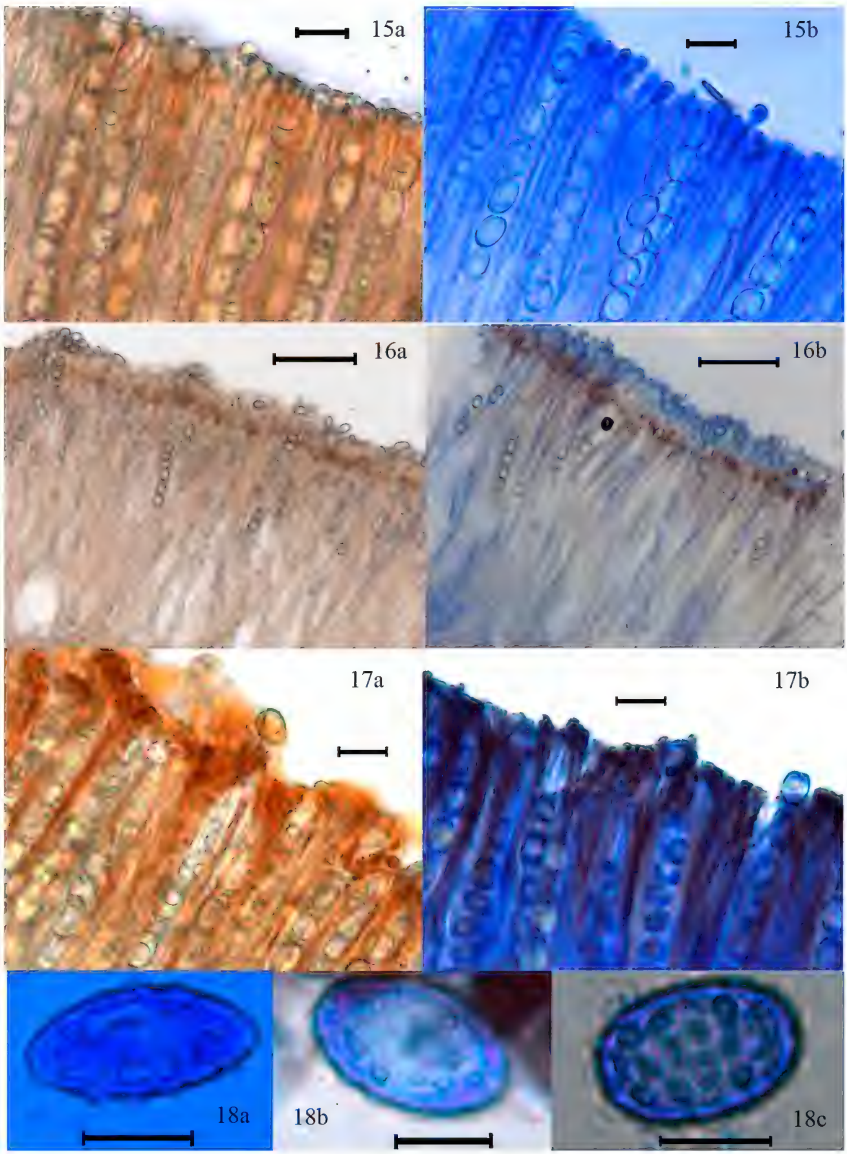
As Baral (1987) noted, we know little about hemiamyloid reaction, and there indeed are few records of this reaction in tissues other than the hymenium. We report this reaction in different apothecial and stipe tissues for *H. leucopus* var. *populina*, *H. robusta*, and *H. subglabra;* we have also observed this reaction in specimens of *H. albella* Quél., *H. connivens* Dissing & M. Lange (holotype), *H. elastica* Bull., and *H. stevensii* Peck (unpublished data).

Concerning ascospore ornamentation, we make the following points. 1) Ornamented ascospores are not restricted to *H.* subgen. *Macropodes* (here represented only by *H. macropus*, Fig. 18a) as Abbott & Currah (1997) proposed. We have also observed ornamented ascospores (generally only in mature ascospores outside the asci) in the type specimens of *H. leucopus* var. *populina* (Fig. 18b), *H. paraphysitorquata* (Fig. 18c), *H. solitaria, H. subglabra*, and *H. ulvinenii*. 2) We agree with Weber (1972) that it is the mature *Helvella* ascospores that are verrucose, contrary to the assumption of Dissing (1964) and Dissing & Nannfeldt (1966) that ornamented ascospores are the immature ones. 3) When citing ascospore sizes, it is important to indicate what kind of spores (smooth vs. ornamented) were measured, because in some species they have different size range (Fig. 9) (see remarks under *H. queletii*).

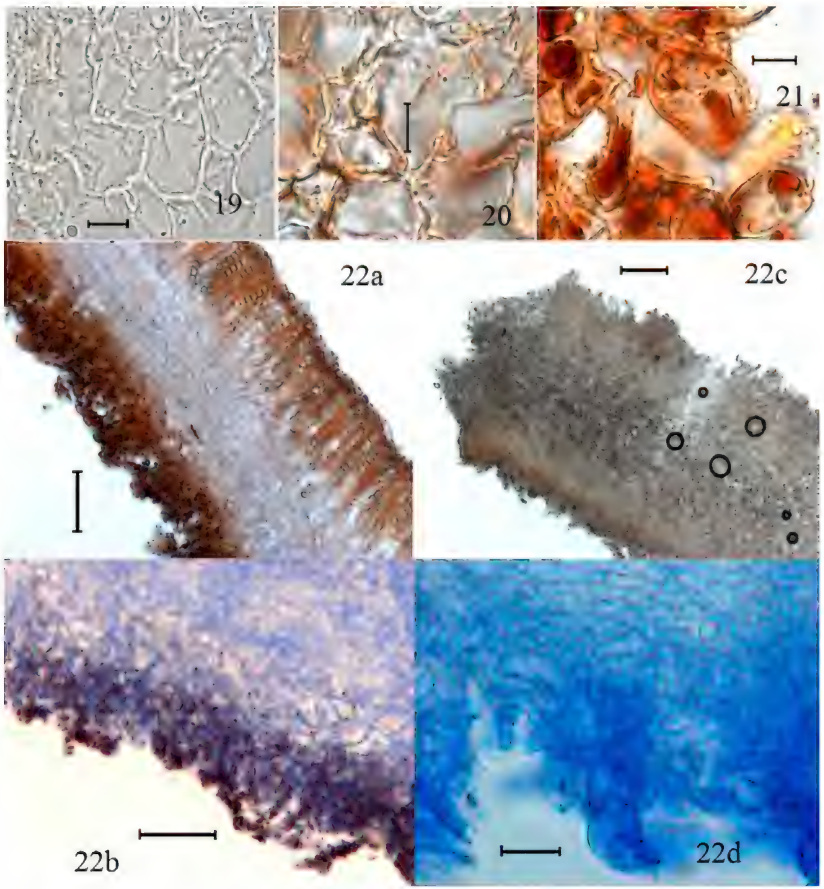
As noted previously, many different terms have been used to describe the apothecial and stipe outer layer surfaces, giving the impression that there were different types of hairs. However, in all cases these hairs are developmentally the same: they consist of hyphal clusters that differ only in length. The tiny hairs have been especially problematic; Weber (1972) and Abbott & Currah (1997)



FIGS. 10–14. 10: Reaction of the apothecial excipulum to Melzer's reagent—a: negative (*Helvella solitaria*), b: hemiamyloid in medullary excipulum (*H. robusta*). 11: Reaction of stipe tissues to Melzer's reagent—a: negative (*H. hyperborea*), b: hemiamyloid in inner layer (*H. subglabra*). 12: Apothecium hyphal fascicles (*H. costifera*)—a: brown pigments visible in water, b) pigments not visible in cotton blue. 13: Apothecium hyphal fascicles (*H. griseoalba*)—a: only terminal cells are pigmented, b: pigments visible in cotton blue. 14: Apothecium hyphal fascicles (*H. dryadophila*)—a: brown pigments visible in water, b: brown pigments visible in cotton blue. Scale bars: 10a, 11a, 11b = 200 µm, 10b = 100 µm, 12–14 = 20 µm.



Figs. 15–18. 15: Paraphyses of *Helvella costifera*—a: brown pigments visible in water, b: pigments not visible under cotton blue. 16: Paraphyses of *H. paraphysitorquata*—a: brown pigments visible only in the thickened apices in water, b: pigments visible in cotton blue. 17: Paraphyses of *H. dryadophila*—a: brown pigments visible in water, b: pigments visible in cotton blue. 18: Ornamented ascospores in cotton blue—a: *H. macropus*, b: *H. leucopus* var. *populina*, c: *H. paraphysitorquata*. Scale bar: 15, 17 = 20 µm, 16a = 100 µm, 18 = 10 µm.



Figs. 19–22. 19: Hyaline ectal excipulum cells in water (*Helvella griseoalba*). 20: Wall pigments in ectal excipulum cells (*H. hyperborea*). 21: Wall and cytoplasmic pigments in ectal excipulum cells (*H. dryadophila*). 22: *H. ulvinenii*—a: apothecium thin section in water, b: ectal excipulum in cotton blue, notice the brown pigments, c: stipe layers in water, d: stipe outer layer under cotton blue. Scale bar: 19–21 = 10 µm, 22a, 22b = 100 µm, 22c = 200 µm, 22d = 20 µm.

used indistinct terms such as "finely pubescent," "furfuraceous," "granulose," or "granulose-roughened" — all with very different meaning. Here we propose to the term "subpubescent" to indicate that they have the same microscopic structure (i.e. are hyphal fascicles) of the hairs found on a "pubescent" surface but are simply shorter (Fig. 2). Likewise, longer hairs have been described as "pubescent" or "villose," without considering that the terms may refer to different types of hairs. We use "pubescent" (Fig. 3) to emphasize that *Helvella* has only one hair type that varies only in length. This agrees with Kaushal's (1991) terminology.

Reasons to retain *H. subglabra* as independent of *H. atra* and *H. griseoalba* separate from *H. costifera* are discussed below. We agree with Harmaja (1977a) that *H. queletii* is a synonym of *H. solitaria* and with Abbott & Currah (1997) that *H. dryadophila* is a synonym of *H. verruculosa*. We also propose *H. hyperborea* as a synonym of *H. costifera*.

We present below a synoptic key of the 12 *Helvella* species considered in this paper. Although not all *Helvella* species are included, we feel that this key provides additional morphological insights into the treated species and contributes to a better understanding of the genus.

Synoptic key to species of *Helvella* included in this work

1) *H. costifera* (= *H. hyperborea*)
2) *H. crassitunicata*
3) *H. griseoalba*
4) *H. leucopus* var. *populina*
5) *H. maculata*
6) *H. paraphysitorquata*
7) *H. pocillum*
8) *H. robusta*
9) *H. solitaria* (= *H. queletii*)
10) *H. subglabra*
11) *H. ulvinenii*
12) *H. verruculosa* (= *H. dryadophila*)

Apothecium shape (mature)
- a. Cup-shaped 1, 2, 3, 7, 8, 9, 11, 12
- b. Lobed 4, 5, 6, 8, 10

Apothecial sterile surface
- a. Glabrous 2, 4
- b. Subpubescent (hyphal fascicles < 50 µm long) 2, 7, 8, 9, 10, 11, 12
- c. Pubescent, with hyphal fascicles 50–150 µm long 1, 3, 5, 6, 7, 8, 9, 11, 12
- d. Pubescent, with hyphal fascicles > 150 µm long 1, 5, 6

Ribs (apothecial sterile surface)
- a. Missing or reaching up to ¼ of the surface 2, 4, 6, 7, 9, 10, 11, 12
- b. Reaching from ¼ to ¾ of the surface 1, 3
- c. Reaching from ¾ to the edge of the apothecium 1, 5, 8
- d. Simple 1, 5
- e. Bifurcated 1, 3, 5, 8
- f. Anastomosed 1, 8

Stipe (surface configuration)
- a. Even 4, 10
- b. Only ribbed 1, 2, 3, 7, 9, 11, 12
- c. Ribbed and lacunose 5, 8
- d. Consisting of 2–3 strands free or apically partly fused 6

Rib (stipe)
- a. Sharp-edged 5, 8
- b. Blunt-edged 1, 2, 3, 7, 9, 11, 12

Stipe (sterile surface)
- a. Glabrous 2, 3, 4
- b. Subpubescent (hyphal fascicles < 50 µm long) 2, 3, 8, 9, 10, 12
- c. Pubescent (hyphal fascicles 50–150 µm long) 1, 5, 6, 7, 8, 9, 11, 12

Stipe color
- a. Light tones 1, 2, 3, 5, 6, 7, 8, 9, 10, 11, 12
- b. Dark tones 4

Stipe context
- a. Solid 1, 2, 3, 6, 7, 9, 10, 11, 12
- b. Hollow 4, 5, 8

Ascus type
- a. Aporhynchous 2
- b. Pleurorhynchous 1, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12

Ascospore size
- a. Usually < 23 µm long 1, 3, 4, 5, 6, 8, 9, 10, 11, 12
- b. Usually > 23 µm long 2, 7

Thick-walled paraphyses
- a. Absent 1, 3, 4, 5, 7, 8, 9, 10, 11, 12
- b. Present 2
- c. Thickness restricted to the apex, forming a collar or hood 6

Melzer's reaction (medullary excipulum)
- a. Negative 1, 2, 3, 4, 5, 6, 7, 9, 11, 12
- b. Hemiamyloid (reddish) 8, 10

Meltzer's reaction (stipe inner layer)
- a. Negative 1, 2, 3, 5, 6, 7, 9, 11, 12
- b. Hemiamyloid (reddish) 4, 8, 10

Melzer's reaction (stipe outer layer)
- a. Negative 1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12
- b. Hemiamyloid (reddish) 8

Paraphyses (pigment topography)
- a. Absent 3
- b. In the cell wall 1, 7, 8, 10, 11
- c. In the cytoplasm 1, 2, 4, 5, 7, 9, 12
- d. Restricted to the apical collar or hood 6

Paraphyses (pigment in cotton blue)
- a. Not visible 1, 5, 8, 10
- b. Visible in all 2, 4, 7, 9, 11, 12
- c. Visible only in thickened apices 6

Pigment (ectal excipulum)
a. Absent 3, 4
b. In the cell wall 1, 2, 5, 6, 7, 8, 9, 10, 11, 12
c. In the cytoplasm 2, 5, 7, 9, 11, 12

Pigment (ectal excipulum in cotton blue)
a. Not visible 1, 5, 6, 8, 9, 10
b. Visible 2, 7, 11, 12

Pigment (stipe outer layer)
a. Absent 1, 3, 11
b. In the cell wall 2, 5, 6, 7, 8, 9, 10, 12
c. In the cytoplasm 2, 4, 5, 12

Pigment (stipe outer layer in cotton blue)
a. Not visible 2, 4, 6, 7, 8, 9, 10, 12
b. Visible in the cytoplasm 5

Pigment (apothecial hyphal fascicles)
a. Absent 5
b. In the cell wall 1, 2, 6, 8, 9, 10, 12
c. In the cytoplasm 2, 7, 11, 12
d. Restricted to the terminal cells (in the wall cell and cytoplasm) 3

Pigment (apothecial hyphal fascicles in cotton blue)
a. Not visible 1, 6, 8, 9, 10
b. Visible in the cytoplasm 7, 11, 12
c. Visible only in the cytoplasm in apical cells 3

Pigment (stipe hyphal fascicles)
a. Absent 1, 3, 5, 11
b. In the cell wall 2, 6, 7, 8, 9, 10, 12
c. In the cytoplasm 2, 12

Taxonomy of type specimens

Helvella costifera Nannf., in Lundell & Nannfeldt, Fungi Exsicc. Suec., Fasc. 41–42: 37, 1953 Figs 3, 12, 15, 23

≡ *Peziza costata* Fr., Nova Acta Reg. Soc. Sci. Uppsal., Ser. 3, 1: 120, 1851

Type: Sweden: Uppland, Uppsala, Botanic Garden, E.P. Fries s.n. *Peziza costata* (Lectotype UPS [not seen]; isolectotypes S [studied], K [not seen]).

= *Helvella hyperborea* Harmaja, Karstenia 18: 57, 1978

Asci 280–370 × 14–18 µm. Paraphyses 4.5–7.5 µm wide at the apex, thin-walled, hyaline, pale brown to brown in mass, pigment deposits in the cytoplasm and cell walls, few with brown pigment encrusted on the wall. Medullary excipulum hyaline to light brown. Ectal excipulum light brown, pigment deposits in the cell walls. Stipe inner and outer layers hyaline. Apothecial hyphal fascicles 100–230 µm long, light brown in mass, pigment deposits in the cell walls; stipe hyphal fascicles 50–120 µm long, hyaline. The brown

pigments of the ectal excipulum are not visible in cotton blue or Melzer's while those of the paraphysis cell walls are visible in Melzer's, but inconspicuous in cotton blue. All tissues rr–.

Additional specimens examined: **FINLAND**: **Lapland**, Enontekiö, 6 Aug 1985, J. Vauras 1932 (H); **Northern Ostrobothnia**, Kuusamo, 4 Aug 1994, T. Ulvinen FO23453 (OULU); **Uusimaa**, Lohja, 19 Jul 1998, U. Nummela-Salo & P. Salo 4924 (H). **NORWAY**: **Northern Norway**, Finnmark, 19 Aug 1995, I. Kylövuori 95-744 (H). **SWEDEN**: **Uppland**, 22 Jun 1948, H. Smith (UPS); 7 July 1948, A. Melderis (UPS); 9 Jul 1948, Exsiccata J.A. Nannfeldt no. 9956 (K).

Remarks — When Nannfeldt (Lundell & Nannfeldt 1953) transferred *Peziza costata* to *Helvella*, he had to publish a nom. nov., *H. costifera*, because the epithet "*costata*" was already occupied in *Helvella* by *H. costata* Schwein. In the protologue of the replaced synonym *Peziza costata*, Fries (1851: 120) had presented two syntypes:

> "In Ohio Americae borealis (*Lindblom*) — etiam copiose in Horto Botanico Upsaliensi una cum *Pez. Helvelloidis* var. minori, *P. sepulta* (Cfr. Summ. Veg. Sc.) *Augusto* pluvio. *El. Fries*, filius [= Elias Petrus Fries]. … Descripti ad specimina Upsaliensia viva, a quibus exsiccatis Fungus Ohioensis non distinguendus."

Nannfeldt (1937: 64) noted that "*Peziza costata* Fr. is represented in the Uppsala Museum by beautiful authentical material," but Lundell & Nannfeldt (1953) cited none of Fries's specimens, noting only that the North American specimen cited by Fries is lost. Subsequently, a UPS syntype specimen collected by E.P. Fries was designated as **lectotype** by Dissing (1966b, as "holotype"), and accepted by Harmaja (1979, as "lectotype?") and Abbott & Currah (1997, as "holotype") — these errors in type terminology are correctable (McNeill et al. 2006: Art. 9.8).

The lectotype was not available for study because of the bad condition of the specimen at UPS. Dissing (1966b) described this species with narrower asci (12–15 µm) and paraphyses (3–4 µm) than we observed. Our isolectotype measurements are closer to those made by Abbott & Currah (1997; asci 313–381 × 13.3–17.1 µm, paraphyses 3.6–6.2 µm at the apex). *Helvella costifera* differs from *H. acetabulum* (L.) Quél. in being less ribbed, with blunt edged ribs, a non-lacunose stipe, grayish hymenium, and pubescent apothecium. In *H. acetabulum* the stipe is highly ribbed to lacunose with sharp edged ribs (Dissing 1966b), the hymenium has brownish tones (Weber 1972), and the sterile part of the apothecium is subpubescent to pubescent. See also discussion under *H. griseoalba* and *H. hyperborea*.

Helvella crassitunicata N.S. Weber, Beih. Nova Hedwigia 51: 30, 1975 Figs 5, 8, 24

Type: U.S.A.: Washington, Mount Rainier National Park, Narada Falls, on soil along a path, 10 Aug 1948, A.H. Smith 30052 (Holotype MICH, Barcode 11561).

Apothecium sterile surface subpubescent. Stipe glabrous to subpubescent. Asci 290–340 × 18–24 µm. Ascospores (21.5–)23–26(–28) × (12–)13–14.5 (–15.5) µm, ellipsoid to oblong, smooth. Paraphyses of two types: a) 5–7.5 µm wide at the apex, thin-walled, septate, hyaline or light brown; b) 7–9 µm wide at the apex, thick-walled, non-septate, light brown, pigment deposits always in the cytoplasm. Ectal excipulum brown, pigment deposits in the cell wall and cytoplasm. Stipe outer layer slightly lighter than the ectal excipulum of the apothecium, pigment deposits in the cell wall and cytoplasm. Apothecial hyphal fascicles ≤ 50 µm long, brown pigment deposits in cell walls and cytoplasm; stipe hyphal fascicles ≤ 10 µm long, tan pigment deposits in cell walls and cytoplasm. The brown pigments of the paraphyses, ectal excipulum, and apothecial hyphal fascicles are visible in cotton blue or Melzer's; light brown pigments of the stipe outer layer and hyphal fascicles inconspicuous in cotton blue or Melzer's. All tissues rr–.

Remarks — *Helvella crassitunicata* and *H. leucomelaena* (Pers.) Nannf. are the only two taxa with aporhynchous asci (Fig. 5) (Abbott & Currah 1997). *Helvella leucomelaena* is differentiated by mature paraphyses that are never thick-walled, slightly smaller (20–23(–25) × 10.5–14 µm) ascospores, and ascomata that fruit in spring or early summer. [However, although Weber (1972) cited *H. crassitunicata* as fruiting in the fall, Abbott & Currah (1997) noted it might fruit from May to October.] Also in *H. leucomelaena* the stipe is poorly developed or absent and the color of the paraphyses is lost in cotton blue.

Neither Weber (1972) nor we observed the following features cited for *H. crassitunicata* by Abbott & Currah (1997): 1) ectal excipulum "pubescent to densely pubescent", 2) stipe "finely pubescent to pubescent", and 3) asci 350–400 × 17–20 µm. It is uncertain whether the differences in wall thickness of the paraphyses should be interpreted as dimorphic (Abbott & Currah 1997) or developmental (young = thin-walled and mature = thick-walled; Weber 1975). Since a hymenium develops first as a palisade of paraphyses within which the asci develop, it would be difficult to have mature asci and immature paraphyses at the same time (Pfister pers. com.). On the other hand, we observed thin and thick-walled branches borne on the same paraphyses. See also remarks under *H. pocillum*.

Helvella dryadophila Harmaja, Karstenia 17: 58, 1977 Figs 14, 17, 21, 25

Type: NORWAY: prov. Oppland, par. Lom, fjled Høyrokampen, alt. 1400–1440 m, *Dryas octopetala* assoc., 29 Aug 1957 F.E. Eckblad, p.p. (Holotype O).

= ***Helvella verruculosa*** (Sacc.) Harmaja, Karstenia 18: 57, 1978

Asci 230–280 × 14–18 µm. Paraphyses 4–7 µm wide at the apex, thin-walled, brown, pigment deposits in the cytoplasm. Medullary excipulum hyaline. Ectal excipulum brown, pigment deposits in the cell wall and cytoplasm.

Stipe inner layer hyaline. Stipe outer layer brown, pigment deposits in cell wall and cytoplasm. Apothecial hyphal fascicles 40–100 µm, brown, pigment deposits in the cell wall and cytoplasm; stipe hyphal fascicles 30–70 µm, brown, pigment deposits in cell walls and cytoplasm. The brown pigments of the paraphyses, ectal excipulum, and apothecial hyphal fascicles are visible in cotton blue or Melzer's; brown pigments of the stipe outer layer and hyphal fascicles inconspicuous in cotton blue. All tissues rr–.

Remarks — We agree with Abbott & Currah (1997) that *H. dryadophila* is a synonym of *H. verruculosa*, because Harmaja (1977b, 1979) used variable characters to distinguish them. 1) Both ascomata appear the same: Harmaja's own illustration (1977b: 51) (Fig. 5) shows both species with a cup-shaped apothecium and a ribbed stipe with ribs that never reach the apothecial sterile surface. 2) For the excipulum, Harmaja cited, "the outermost layer has somewhat larger cells with slightly thicker wall as *H. arctoalpina,*" but gave no measurements, and we did not see such differences. 3) The paler and homogeneous contents of paraphyses in *H. dryadophila* did not appear paler to us; in fact, in cotton blue, the brown pigments are visible in the paraphyses of both species. The homogeneous content is variable and the pigment tends to be less homogeneous in immature ascomata. 4) As the presence of a fairly distinct layer of textura angularis in the excipulum is a generic character for *Helvella*, its occurrence should not be used to distinguish between species.

Abbott & Currah (1997) mentioned that *H. arctoalpina* Harmaja (Harmaja 1977) should also be considered a synonym of *H. verruculosa*, but as the type collection of *H. arctoalpina* is not in O, we unfortunately could not examine the holotype.

Helvella griseoalba N.S. Weber, Michigan Bot. 11: 162, 1972 Figs 2, 13, 19, 26

Type: U.S.A.: Michigan, Cheboygan Co., Grapevine Point, Douglas Lake, Univ. of Michigan Biol. Sta., 10 Jun 1968, N.J. Smith 982 (Holotype MICH, Barcode 14379).

Asci 220–265 × 12.5–16 µm. Medullary & ectal excipula hyaline. Stipe inner and outer layers hyaline. Apothecial hyphal fascicles 70–120 µm long, hyaline with brown terminal cells, pigment deposits in the cell wall and cytoplasm; stipe hyphal fascicles ≤ 30 µm long, completely hyaline. The brown pigments of terminal cells of the apothecial hyphal fascicles are visible in cotton blue or Melzer's. All tissues rr–.

Remarks — Although in fresh ascomata the hymenium and sterile surface of the apothecium are concolorous (gray to cinnamon; Weber 1972), the dried sterile surface is lighter, cream to light brown, and the hymenium is slightly darker, grayish brown. This may be one reason why dry *H. griseoalba* material can be easily confused with *H. costifera*. Both Häffner (1987) and Abbott & Currah (1997) synonymized *H. griseoalba* under *H. costifera,* noting that color

differences of the hymenial surface and absence of pigments in the apothecial ectal excipulum in *H. griseoalba* were not enough to separate them. However, we observed additional features in the types of *H. costifera* and *H. griseoalba* that support keeping them as different taxa: 1) *H. costifera* presents pubescent hyphal fascicles in the stipe while *H. griseoalba* does not (glabrous) or has shorter (subpubescent) hyphal fascicles; 2) *H. costifera* can have light brown paraphyses while *H. griseoalba* has only hyaline ones, and 3) in *H. griseoalba* the terminal cells of the apothecial hyphal fascicles have brown pigments that are visible in cotton blue (Fig. 13) while in *H. costifera* the light brown pigments in the cell walls are not visible in cotton blue (Fig. 12).

Helvella hyperborea Harmaja, Karstenia 18: 57, 1978 Figs 11a, 20, 27

Type: **FINLAND**: prov. Kuusamo, par. Kuusamo, Juuma, western part of the gorge Jäkälävuoma, alt. ca. 205 m, on a shady shelf in the basal part of a steep dolomitic rock, among the moss *Distichium capillaceum* (etc.), accompanied by *Salix reticulata, Saxifraga aizoides, S. nivalis, Woodsia glabella, Gerronema albidum*, 27 Aug 1970, H. Harmaja (Holotype H).

= ***Helvella costifera*** Nannf., in Lundell & Nannfeldt, Fungi Exsicc. Suec., Fasc. 41–42: 37, 1953

Asci 240–315 × 15–18 µm. Paraphyses thin-walled, hyaline, pale brown to brown in mass, pigment deposits in cytoplasm and cell walls, some with brown encrusted pigment on walls. Medullary excipulum hyaline to light brown. Stipe inner and outer layers hyaline. Apothecial hyphal fascicles 50–160 µm long, light brown in mass, pigment deposits in cell walls; stipe hyphal fascicles 50–100 µm long, hyaline. The brown pigments of all structures are not visible in cotton blue. All tissues rr–.

Additional specimens examined: **FINLAND**: **Lapland,** Kemi, 7 Aug 1998, U. Nummela-Salo & P. Salo 5318 (H); **Nortern Savonia**, Kuopio, 22 July 1984, J. Vauras 1638F (H); 23 Aug 1987, J. Vauras 2856 (H); **Varsinais-Suomen**, 4 Jul 1996, J. Vauras 11192F (H). **NORWAY**: **Northern Norway,** Troms, 16 Aug 1992, I. Kytövuori 92-352 (H).

Remarks — *Helvella hyperborea* was proposed as a new species by Harmaja (1978) and accepted as distinct by Abbott & Currah (1997). After comparing its type with *H. costifera,* including eleven additional specimens from Herbaria O and UPS (labeled either *H. costifera* or *H. hyperborea*), we conclude these two species are the same. Harmaja (1979, Table 1) listed 12 characters for differentiating *H. hyperborea* from *H. costifera* but explained that only a combination of all features should be used for its identification. In fact, we think that his table is an excellent presentation of variability in *H. costifera*. Abbott & Currah (1997) used only the ribs of the apothecial sterile surface to separate the species, distinguishing *H. costifera* by branched anastomosed ribs extending up to the marginal area from *H. hyperborea* with simple unbranched

ribs extending onto the basal half. However, both isolectotype and exsiccata (J.A. Nannfeldt no. 9956) of *H. costifera* possess the same features used to identify *H. hyperborea* (see Figs 23, 27).

We provide below our evaluation of what we consider the five most important features used by Harmaja (1979) to separate these species.

1) A brown-grey hymenium color that is paler in *H. costifera* — we have observed that in fresh material, the color intensity of the hymenium may depend on whether the specimen is growing in a forest clearing or in a shaded area.

2) Ribs extending onto the sterile surface, especially in dried specimens — Although Harmaja (1979; Figs. 2–3) illustrated this feature, we found that the *H. costifera* isolectotype also has ribs on the sterile surface as, for that matter, does *H. acetabulum*.

3) Sterile surface of the apothecium: "with fine but almost always ± distinct hyaline to pale brown pubescence or villosity" (*H. costifera*) versus "more or less delicate brown (mostly dark) pubescence usually appearing glabrous to bare eye" (*H. hyperborea*) — Under the microscope, the hairs of all studied specimens have the same color. However, we measured 100–230 µm long hyphal fascicles in the *H. costifera* type vs. 50–160 µm long fascicles in *H. hyperborea*. The hairs as measured in the additional specimens range from 50–240 µm long, depending on the maturity of the ascoma. In the stipe hair lengths are similar for both types — 50–120 µm long in *H. costifera*, 40–100 µm long in *H. hyperborea* — and remains relatively constant (40–120 µm) in the additional specimens.

4) Pigmentation of the paraphyses: "medium (rarely pale) brown wall encrustation" and more or less brown contents (*H. hyperborea*) versus hyaline, inconspicuous or pale brown encrustation and pale brown to practically hyaline contents (*H. costifera*) — We observed the same color in the paraphyses for both species, and in cotton blue the cytoplasmic pigment is inconspicuous; the cell wall pigment is variable among specimens in both species.

5) Distribution, "middle boreal to lower oroarctic (low alpine), optimal area apparently northern boreal zone" (*H. hyperborea*) versus "temperate to middle (oro)boreal with preference for southern and low-lying areas" (*H. costifera*) — Sequences of the 28s large subunit ribosomal DNA from three specimens identified as *H. costifera* (unpublished) from Scandinavia (two from northern boreal specimens and one from the middle boreal region) show only one or two base differences. Notably, both species were described from the Scandinavian peninsula.

Helvella leucopus* var. *populina I. Arroyo & Calonge, in Calonge, Bol. Soc. Micol. Madrid 25: 302, 2000 Figs 1, 18b, 28

Type: Spain: Guadalajara, Sigüenza, 8 May 1988, M.J. Rodríguez 888 (Holotype MA Fungus 22870).

Apothecium sterile surface glabrous, even. Stipe tapering toward the apex, hollow, glabrous. Asci pleurorhynchous. Ascospores verrucose, smooth within the ascus, hyaline, uniguttulate. Paraphyses 3.5–4.5 µm wide at the apex, thin-

walled, hyaline or brown, pigment deposits in the cytoplasm. MEDULLARY AND ECTAL EXCIPULA hyaline. STIPE INNER LAYER hyaline. STIPE OUTER LAYER light brown, pigment deposits in the cytoplasm. HYPHAL FASCICLES absent in the apothecium and stipe. The brown pigments of the paraphyses are visible and those of the stipe outer layer are not visible in cotton blue or Melzer's. Stipe inner layer rr+; medullary and ectal excipula and stipe outer layer rr–.

REMARKS — Persoon (1822) described *H. leucopus* Pers. as having a deflexed apothecium at both ends (lobate), bay to black in color, and a 30–40 mm long glabrous white stipe. Arroyo & Calonge (1990) distinguished variety *populina* (validated by Calonge 2000) by the darker stipe color and larger ascoma size (apothecium 40–60 × 30–45 mm; stipe 50–120 × 15–35 mm). However, Moravec (1980) previously cited larger specimens of *H. leucopus* var. *leucopus* (ascomata ≤ 150 mm high) from Slovakia. Ascoma size can be quite variable and so should be considered with caution; for example, *H. crispa* (Scop.) Fr. and *H. lacunosa* ascomata may range from 50 to 200 mm high. Arroyo & Calonge (1990) described smooth ascospores and ≤ 12 µm wide paraphyses apices, while we observed verrucose mature ascospores (more evident in cotton blue or Melzer's) and narrower paraphyses.

Helvella maculata N.S. Weber, Beih. Nova Hedwigia 51: 27, 1975 FIG. 29

TYPE: U.S.A.: Idaho, Bonner Co., south side of Hoodoo Mountain, 5 Oct 1968, H.V. Smith et N.J. Smith (N.J. Smith 2124) (Holotype MICH, Barcode 5635).

APOTHECIUM sterile surface pubescent. ASCOSPORES 18–22.5 × 10.5–12 µm. ECTAL EXCIPULUM yellowish brown, pigment deposits in cell walls and cytoplasm. STIPE INNER LAYER hyaline. STIPE OUTER LAYER brown, pigment deposits in cell walls and cytoplasm. APOTHECIAL HYPHAL FASCICLES 107–205 µm long, hyaline; STIPE HYPHAL FASCICLES 60–130 µm long, hyaline. The brown pigments of the paraphyses and ectal excipulum of the apothecium are inconspicuous and those of the stipe outer layer are very conspicuous in cotton blue or Melzer's. All tissues rr–.

REMARKS — We measured smaller ascospores than those cited by Weber (1975, 20–23 × 12–13.5 µm). *Helvella maculata* might be confused with *H. crispa*, but the mottled apothecium, grayish stipe ribs, pubescent sterile surface, and incurved and unrolling margin of *H. maculata* are diagnostic (Weber 1975; Abbott & Currah 1997). Furthermore, the brownish pigment of the stipe outer layer is evident in cotton blue in *H. maculata* but not in *H. crispa*. For Weber (1975) ascospore size could help differentiate the two species, but the size range we observed for *H. maculata* overlaps with that seen in *H. crispa* (16–20.5 × 9.8–12 µm). Another similar species, *H. fusca* Gillet, has a dark red-brown hymenium, distinctly ribbed sterile surface with some ribs reaching the apothecium margin, and vernal fructification associated with *Populus*,

Figs. 23–29: Ascomata of type specimens (except when indicated) of *Helvella*. 23: *H. costifera* (Exsiccata J.A. Nannfeldt no. 9956). 24: *H. crassitunicata*. 25: *H. dryadophila*. 26: *H. griseoalba*. 27: *H. hyperborea*. 28: *H. leucopus* var. *populina*. 29: *H. maculata*. Scale bar: 1 cm.

while *H. maculata* fruits in autumn and is associated with conifers (Weber 1975); furthermore, the apothecium sterile surface of *H. fusca* is subpubescent. According to Abbott & Currah (1997), *H. maculata* could also be confused with *H. lacunosa*, which differs in having an apothecium margin attached to the stipe, black hymenium, and glabrous sterile surface of apothecium and stipe.

Helvella paraphysitorquata I. Arroyo & Calonge, in Calonge & Arroyo, Mycotaxon 39: 210, 1990 FIGS 7, 16, 18c, 30

TYPE: Spain: within the province of Teruel, Albarracín, growing in soil under *Populus*, 3 Jun 1988, I. Arroyo & F.D. Calonge (Holotype MA-Fungi 24512).

APOTHECIUM sterile surface pubescent, even. STIPE pubescent. ASCI pleurorhynchous. ASCOSPORES 16.5–19 × 11–13.5 µm, ovoid to ellipsoid, verrucose, smooth within the ascus. PARAPHYSES 5–9 µm wide at the apex, thin-walled, hyaline, with a brown thick-walled cap or collar at the apex. MEDULLARY EXCIPULUM hyaline. ECTAL EXCIPULUM hyaline to light brown, pigment deposits in cell walls. STIPE INNER AND OUTER LAYERS hyaline to light brown, pigment deposits in cell walls. APOTHECIAL HYPHAL FASCICLES 60–220 µm long, hyaline to light brown in mass, pigment deposits in the cell wall; STIPE HYPHAL FASCICLES 60–140 µm, hyaline to light brown in mass, pigment deposits in the cell wall. The brown pigments of the paraphyses are visible in cotton blue, and pigments of the ectal excipulum, apothecial hyphal fascicles, and stipe outer layer and hyphal fascicles are not visible in cotton blue or Melzer's. All tissues rr–.

REMARKS — Calonge & Arroyo (1990) described *H. paraphysitorquata* with smooth ascospores, but we also found ornamented ascospores on the hymenial surface, stipe, and basal mycelium. We observed narrower paraphyses than those recorded by Calonge & Arroyo (1990; 7–10(–15) µm), possibly due to their rehydration in 2% KOH or 10% NH_3. *Helvella paraphysitorquata* is morphologically similar to *H. pezizoides* Afzel., which differs in its totally black even stipe and paraphyses lacking the brown collar. On the other hand, in a specimen determined as *H. macropus* from Mexico (J.M. Rodríguez Canseco 12, IBUG), we also observed a few or occasional brown capped or collared paraphyses, so apparently collared paraphyses are not unique to *H. paraphysitorquata*.

Helvella pocillum Harmaja, Karstenia 15: 30, 1976 FIGS 4, 31

TYPE: Sweden: prov. Torne Lappmark, par. Jukkasjärvi, fjeld Låktatjåkko, on bare soil, 17 Aug 1946, L. Holm 472 (Holotype UPS).

APOTHECIUM sterile surface subpubescent to pubescent, concolorous with the hymenial surface, stipe ribs only reaching the apothecium basis. STIPE 1–5 × 1–3 mm, solid, costate, ribs blunt, pubescent, concolorous with the hymenial surface. ASCI 320–380 × 18–24 µm, pleurorhynchous. ASCOSPORES 23–28 × 12–15.5 µm, smooth. PARAPHYSES thin-walled. MEDULLARY EXCIPULUM hyaline. ECTAL EXCIPULUM brown, pigment deposits in cytoplasm. STIPE INNER LAYER hyaline. STIPE OUTER LAYER light brown, pigment deposits in cell walls. APOTHECIAL HYPHAL FASCICLES 40–100 µm long, brown, pigment deposits in cytoplasm; STIPE HYPHAL FASCICLES 50–80 µm, hyaline to light brown in mass,

pigment deposits in cell walls. The brown pigments of the paraphyses, ectal excipulum, and apothecial hyphal fascicles are visible and those of the stipe outer layer and hyphal fascicles are not visible in cotton blue or Melzer's. All tissues rr–.

Remarks — *Helvella pocillum* is characterized by its very small ascoma; only *H. rivularis* Dissing & Sivertsen is as small, but with an even stipe. For Harmaja (1976) *H. pocillum* could be separated from *H. crassitunicata* by its larger ascospores, the width of the paraphyses (we found no differences in either of these characters), and the absence of thick-walled paraphyses. Besides, *H. crassitunicata* has aporhynchous asci (Fig. 5) and the sterile surfaces of apothecium and stipe are glabrous to subpubescent. Another similar species is *H. leucomelaena*, also with aporhynchous asci.

Helvella queletii Bres., Fungi trident. 1(3): 39, 1882 Fig. 32

Type: Italy: Bosee di lance sopra Terzolaj, May 1882, J. Bresadola (Holotype S).

= ***Helvella solitaria*** P. Karst., Bidr. Känn. Finl. Nat. Folk 19: 37, 1871

Stipe solid, costate, ribs blunt. Asci 260–310 × 15–20 µm, pleurorhynchous. Ascospores 17.5–21.5 × 10.5–12.5 µm, broadly ellipsoidal, smooth. Paraphyses 3.5–7 µm wide at the apex, thin-walled, brown, pigment deposits in the cytoplasm. Medullary excipulum hyaline. Ectal excipulum brown, pigment deposits in cell walls and cytoplasm. Stipe inner layer hyaline. Stipe outer layer light brown, pigment deposits in cell walls. Apothecial hyphal fascicles 25–60 µm long, light brown in mass, pigment deposits in the cell wall; stipe hyphal fascicles 40–100 µm, light brown, pigment deposits in cell walls. The brown pigments of the paraphyses are visible and pigments of the ectal excipulum, stipe outer layer, and apothecial and stipe hyphal fascicles are not visible in cotton blue or Melzer's. All tissues rr–.

Additional specimens examined: **SPAIN**: **Madrid**, Madrid, 7 Apr 2001, A. González, J.C. Campos et al., G.P. 1395 (MA-Fungi 73836); **Castile and León**, Valladolid, 31 Mar 2001, Herrera de Duero 1294 (MA Fungi 54822).

Remarks — Bresadola (1882), who described *H. queletii* as having a cup-shaped apothecium, sulcate-lacunose stipe ("costate" according to Weber 1972), and ascospores measuring 17–20 × 12 µm, noted its proximity to *H. lacunosa* but without mentioning the differences with *H. solitaria*. Dissing (1966b) differentiated *H. queletii* from *H. solitaria* by the following: 1) ascospore size — 17–19.1–21 × 11–12.3–13.5 µm (*H. queletii*) vs. 19–21.7–24 × 12–13.1–15 µm (*H. solitaria*); 2) ascoma size — 20–80 mm broad apothecium, 20–60 mm high stipe (*H. queletii*) vs. 10–40 mm broad apothecium, 10–25 mm high stipe (*H. solitaria*); 3) hymenium color (pale brownish to dark greyish-brown (*H. queletii*) vs. greyish to greyish-brown (*H. solitaria*); 4) stipe rib number — 4–7 in *H. queletii* vs. 2–5 in *H. solitaria*; 5) fruiting time — April–October in *H. queletii* vs. February–June in *H. solitaria*.

Harmaja (1977a) suggested that *H. queletii* be recognized as a synonym of *H. solitaria* based on the variability of ascoma size, color, pubescence, and stipe rib number. Abbott & Currah (1997) also considered them synonymous (but see below under *H. solitaria*). The following list summarizes our observations of both holotypes and other original descriptions.

1) The *H. solitaria* holotype has smooth ascospores and a spore range (18–20 × 9.5–12 µm) matching that of the *H. queletii* holotype and as reported by Dissing (1966b). We also found smaller (15–17.5 × 9.5–11.5 µm) verrucose ascospores more closely matching measurements given by Karsten (1871, 14–16 × 10 µm) but never matching that cited by Dissing (1966b).

2) Ascoma size, highly variable in *Helvella,* is not a good taxonomic criterion for a widespread species,

3) Ascoma color is highly variable in *H. queletii* with tones very similar to *H. solitaria,* as noted in Dissing (1966a) and Dissing & Nannfeldt (1966),

4) Among materials cited as *H. queletii* by Dissing (1966b) is a specimen collected in March (Velenovsky s.n., Mar 1923, PRC), while among his *H. solitaria* materials are specimens collected in July (Eftesøl s.n., 8 Jul 1956, O; Berg s.n., 15 Jul 1957, O), August (Bresadola s.n., Aug 1893, S; Hakelier s.n., 28 Aug 1962, UPS), September (P. Karsten s.n., 21 Sep 1866, H), October (Toft s.n., 21 Oct 1965, C), and November (Bresadola s.n., Nov 1896, S; Arwidsson s.n., 1 Nov 1943, S). Thus, the time of fruiting is not diagnostic.

5) Both holotypes are anatomically similar, except that the brown cytoplasmic pigment in the paraphyses is lighter in *H. solitaria*.

Helvella robusta S.P. Abbott, in Abbott & Currah, Mycotaxon 33: 242, 1988

Figs 10b, 33

Type: CANADA: Calgary, Nose Hill area, 30 Aug 1972, R.M. Danielson 459 (Holotype DAOM-143869).

Apothecium irregularly cupulate with a large central depression to irregularly bi-lobed, margin free, covering the stipe; sterile surface subpubescent to pubescent, ribbed, ribs sharp. Stipe gradually expanding toward the apothecium, lacunose, ribs sharp, subpubescent to pubescent. Paraphyses hyaline to light brown, pigment deposits in cell walls. Medullary excipulum hyaline. Ectal excipulum light brown, pigment deposits in cell walls. Stipe inner layer hyaline. Stipe outer layer light brown, pigment deposits in cell walls. Apothecial hyphal fascicles 38–80 µm long, hyaline to light brown, pigment deposits in cell walls; stipe hyphal fascicles 40–90 µm long, hyaline to light brown, pigment deposits in cell walls. The brown pigment of the paraphyses, ectal excipulum, apothecial hyphal fascicles, and stipe outer layer is not visible in cotton blue or Melzer's. Medullary excipulum and stipe inner and outer layers rr+; ectal excipulum rr–.

Remarks — Abbott & Currah (1988, 1997) described *H. robusta* with apothecium that is irregularly cupulate or centrally depressed with reflexed margins; in addition to this type, we observed an irregularly bi-lobed apothecium covering the stipe, with the apothecial edges curved towards the stipe, thus fully exposing the hymenium. The peculiar shape of the ascoma makes *H. robusta* easy to recognize, although Abbott & Currah (1988) noted that it could be confused with some species of section *Acetabulum*, although members of that section have a well-defined cup-shaped apothecium in all stages of development. In addition to its peculiar apothecial shape, *H. robusta* has a strong reddish reaction in the apothecial medullary excipulum and stipe inner layer and moderately reddish reaction in the stipe outer layer in Melzer's.

Helvella solitaria P. Karst., Bidr. Känn. Finl. Nat. Folk 19: 37, 1871 Figs 9, 10a, 34

Type: Finland: Mustiala, in horto, ad marg. rivula, 21 Sep 1866, P. Karsten PAK 3288 (Holotype H).

= *Helvella queletii* Bres., Fungi trident. 1(3): 39, 1883

Ascospores a) smooth: 18–20 × 9.5–12 µm, broadly ellipsoidal; b) verrucose: 15–17.5 × 9.5–11.5 µm, ellipsoidal. Paraphyses 4–7 µm wide at the apex, thin-walled, light brown, pigment deposits in the cytoplasm. Medullary excipulum hyaline. Ectal excipulum brown, pigment deposits in cell walls and cytoplasm. Stipe inner layer hyaline. Stipe outer layer light brown, pigment deposits in cell walls. Apothecial hyphal fascicles 40–90 µm long, light brown, pigment deposits in cell walls; stipe hyphal fascicles 35–90 µm, light brown in mass, pigment deposits in cell walls. The brown pigments of the paraphyses are visible, and pigments of the ectal excipulum, stipe outer layer, and apothecial and stipe hyphal fascicles are not visible in cotton blue or Melzer's. All tissues rr–.

Remarks — *Helvella solitaria* can be confused with *H. pocillum*, *H. ulvinenii,* and *H. verruculosa* because of its cup-shaped apothecium, costate stipe with ribs extending only to the base or a short distance onto the apothecium, and pleurorhynchous asci. *Helvella pocillum* has larger ascospores (22–30 × 13–17 µm). *Helvella ulvinenii* has brown, sepia to black apothecial sterile surface (never with grayish tones), brown pigment of the apothecial hyphal fascicles visible in cotton blue and hyaline stipe hyphal fascicles. *Helvella verruculosa* differs in the visibility of the brown cytoplasmic pigments in the ectal excipulum and apothecial hyphal fascicles in cotton blue, and its arctic and alpine distribution contrasts with the boreal and temperate distribution for *H. solitaria* (Abbott & Currah 1997).

Paraphysis widths cited here match those by Karsten (1871) but not those by Abbott & Currah (1997), who cited paraphyses with 7–8 µm wide apices. Karsten (1871) gave 14–16 × 10 µm for *H. solitaria* ascospores but did not

mention whether they were smooth or ornamented. We agree with Harmaja (1977a,b) that mature and immature ascospore sizes differ in some species, but in view of the greater size variation in *H. solitaria*, we consider it important to list the sizes of both immature and mature ascospores.

Although Dissing (1966b) recognized *H. solitaria* and *H. queletii* as different species, we agree with Harmaja (1977a) that they are synonyms, with the name *H. solitaria* having priority (see discussion under *H. queletii*).

According to Abbott & Currah (1997), *H. solitaria* sensu Dissing (1966b) corresponds to *H. leucomelaena*. We do not agree with this because of the following: 1) *H. leucomelaena* has aporhynchous asci; Dissing (1966b) did not mention the ascus base, and 2) the specimens studied by Dissing do not seem to correspond to a single species. Harmaja (1977a) designated one Dissing *H. solitaria* specimen as holotype of *H. confusa* Harmaja (also considered a synonym under *H. leucomelaena* by Abbott & Currah, 1997), while other Dissing specimens correspond to different species, among them *H. leucomelaena* and *H. solitaria* (Harmaja 1979). As Dissing's concept of *H. solitaria* was very broad, it cannot be attributed to a particular taxon.

Helvella subglabra N.S. Weber, Michigan Bot. 11: 179, 1972 FIGS 6, 11b, 35

TYPE: U.S.A.: Michigan, Washtenaw, Co., Stinchfield Woods, near Dexter, 13 Oct 1968, N.J. Smith 2145 (Holotype MICH, Barcode 14381).

ASCI 224–324 × 15–17 μm, pleurorhynchous. ASCOSPORES 15–19 × 10–11.5 μm, smooth, few verrucose. PARAPHYSES thin-walled, hyaline to pale brown, pigment deposits in the cell wall. STIPE OUTER LAYER brown, pigment deposits in the cell wall. APOTHECIAL & STIPE HYPHAL FASCICLES ≤ 30 μm long, scattered; hyaline to brown, pigment deposits in cell walls. The brown pigments of all structures are not visible in cotton blue. Medullary excipulum and stipe inner layer rr+; ectal excipulum and stipe outer layer rr–.

REMARKS — Häffner (1987) and Abbott & Currah (1997) synonymized *H. subglabra* under *H. atra* J. König. They considered that the differences listed by Weber (1972) were not enough to separate them, because other species (*H. lacunosa*, *H. albella*, *H. latispora* Boud.) also accommodate such variability. Distinctions noted by Weber (1972) are: 1) hymenium surface color— dark gray to brownish gray or drab (*H. subglabra*) vs. black to grayish black (*H. atra*); 2) color of stipe and apothecial sterile surface — drab to gray (*H. subglabra*) vs. black or dark gray (*H. atra*); 3) pigments in cells of the ectal excipulum — scattered hyphal cells with brown walls (*H. subglabra*) vs. most hyphal cells with brown walls (*H. atra*); and 4) apothecial sterile surface texture — subpubescent (*H. subglabra*) vs. completely glabrous (*H. atra*). We add here two more characters that separate *H. subglabra* from *H. atra*: 1) tissues in Melzer's — ascoma negative (*H. atra*) vs. apothecial medullary excipulum

Figs. 30–37: Ascomata of type material of *Helvella*. 30: *H. paraphysitorquata*. 31: *H. pocillum*. 32: *H. queletii*. 33: *H. robusta*. 34: *H. solitaria* (scanned image of the picture contained in the type specimen). 35: *H. subglabra*. 36: *H. ulvinenii*. 37: *H. verruculosa*. Scale bar: 1 cm.

weakly reddish and stipe inner layer strongly reddish (*H. subglabra*); 2) the brown cell wall pigment in the paraphyses, ectal excipulum, and stipe outer layer is visible in cotton blue in *H. atra*.

Helvella ulvinenii Harmaja, Karstenia 19: 42, 1979 Figs 22, 36

Type: Finland: prv. Enontekiön Lappi, par. Enontekiö, Kilpisjärvi, NE slope of W peak of fjeld Pikku-Malla, alt. ca. 650 m, H. Harmaja, 25 Aug 1979 (Holotype H).

Apothecium sterile surface subpubescent to pubescent. Stipe solid, costate, ribs blunt, pubescent. Asci 240–300 × 14–17 µm. Ascospores 14.5–18.5 × 10.5–12(–13.5) µm, broadly ellipsoid, smooth and verrucose. Stipe inner & outer layers hyaline. Apothecial hyphal fascicles 40–105 µm long, brown, pigment deposits in the cytoplasm; stipe hyphal fascicles 60–140 µm, hyaline. The brown pigments of the paraphyses, ectal excipulum, and apothecial hyphal fascicles are visible in cotton blue or Melzer's. All tissues rr–.

Remarks — Harmaja (1979) did not mention ascospore ornamentation for *H. ulvinenii*; we observed both smooth (immature), and verrucose (mature) ascospores with the same size and shape. *Helvella ulvinenii* shares cup-shaped apothecia and costate stipes with ribs that do not reach the sterile apothecial surface with *H. solitaria* and *H. verruculosa*. We agree with Harmaja (1979) that *H. ulvinenii* is distinguished from *H. solitaria* by 1) a darker sterile surface that is never grayish, 2) shorter ascospores, and 3) ectal excipular hyphae with distinctly dark pigments, present only in the cell wall and not in the cytoplasm. Additional distinctions include: 1) the stipe outer layer and hyphal fascicles are hyaline in *H. ulvinenii* and light brownish (on the cell walls) in *H. solitaria*, and 2) the apothecial hyphal fascicle pigments are deposited in the wall in *H. solitaria* and occur both in the cytoplasm and in the cell wall in *H. ulvinenii*.

Harmaja (1979) differentiated *H. verruculosa* (as *H. dryadophila*) from *H. ulvinenii* based on 1) apothecial shape, 2) longer ascospores, and 3) very long paraphysis end cells. Although we did measure longer ascospores (17.5–22 × 10.5–13 µm) for *H. verruculosa*, both types had cup-shaped apothecia and ribbed stipes and the paraphysis end cells appeared the same. In addition, we observed that *H. verruculosa* has pigmented stipe hyphal fascicles. Probably, *H. ulvinenii* is a variety of *H. verruculosa*.

The *H. ulvinenii* type demonstrates particularly well the differences between the structure of the ectal excipulum and apothecial hyphal fascicles and of the stipe outer layer and hyphal fascicles; cells are pigmented in the apothecium and hyaline in the stipe (Fig. 22).

Helvella verruculosa (Sacc.) Harmaja, Karstenia 18: 57, 1978 Fig. 37

Type: Russia: Madagan Obl., Chukotski Peninsula, Arakamchene Island by the Bearing Strait, Stony Hills, 11 Aug ca. 1885, Wright, Herb. U.S.N. Pacif. Expl. Exped. No. 369 (Holotype K, Isotype FH, both studied).

= *Geopyxis verruculosa* Sacc., Sylloge fungorum 8: 68, 1889

= *Helvella dryadophila* Harmaja, Karstenia 17: 58, 1977

Paraphyses 4–8 µm wide at the apex, thin-walled, brown, pigment deposits in the cytoplasm. Medullary excipulum hyaline. Ectal excipulum brown, pigment deposits in cell walls and cytoplasm. Stipe inner layer hyaline.

STIPE OUTER LAYER brown, pigment deposits in cell walls and cytoplasm. APOTHECIAL HYPHAL FASCICLES 70–150 µm, brown, pigment deposits in cell walls and cytoplasm; STIPE HYPHAL FASCICLES 50–130 µm, brown, pigment deposits in cell walls and cytoplasm. The brown pigments of the paraphyses, ectal excipulum, and apothecial hyphal fascicles are visible and those of the stipe outer layer and hyphal fascicles are not visible in cotton blue or Melzer's. All tissues rr–.

REMARKS — Abbott & Currah (1997) described this species with a "villose margin" of the apothecium (sometimes with distinct white hairs), the stipe as having few chambers, and with stipe ribs extending onto basal half of apothecial sterile surface. We did not observe the marginal hairs, perhaps because the hairs have been lost due to age or handling. Furthermore, we observed neither stipe chambers nor ribs on the sterile surface of the apothecium. In fact, *H. verruculosa* is more easily confused with *H. solitaria* and *H. ulvinenii*, which do not have ribs on the sterile surface (see differences under the remarks of these species) than with *H. acetabulum*, *H. costifera*, and *H. griseoalba*.

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Psilocybe s.s. in Thailand: four new species and a review of previously recorded species

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Abstract — *Psilocybe deconicoides*, *P. cubensis*, *P. magnispora*, *P. samuiensis*, and *P. thailandensis* (previously known from Thailand) are revisited, and *P. thaiaerugineomaculans*, *P. thaicordispora*, *P. thaiduplicatocystidiata*, and *P. thaizapoteca* are described as new species. These new species are bluing and belong to sections *Cordisporae*, *Stuntzae*, and *Zapotecorum*. Following the recent conservation of *Psilocybe* as the generic name for bluing species, *P. deconicoides*, which does not blue upon bruising, is transferred to *Deconica*, while the bluing taxa *P. cubensis* (sect. *Cubensae*), *P. magnispora* and *P. thailandensis* (sect. *Neocaledonicae*), and *P. samuiensis* (sect. *Mexicanae*) remain in *Psilocybe*.

Key words — hallucinogenic mushrooms, richness mycobiota, *Strophariaceae*, tropics

Introduction

The hallucinogenic fungal species in Thailand, as in most tropical countries, are poorly known, which is in direct contrast with the large fungal diversity that occurs throughout the tropics. Moreover, with considerable destruction of tropical habitats for use as agricultural or cattle farms, many species will likely disappear before being documented. We have been surveying several mushroom genera in northern Thailand (e.g. Le et al. 2007, Sanmee et al. 2008, Wannathes et al. 2009, Zhao et al. 2010, Karunarathna et al. 2011); but studies of *Psilocybe* are rare. Despite the recreational use of hallucinogenic mushrooms in the country (see Allen & Merlin 1992), we have found only a few references

in papers by Heim (1958, 1962), Guzmán et al. (1993), Chamratpan (2003), Klinhom et al. (2003), and Horak et al. (2009). Fortunately, Thailand now has a mycological research station, Mushroom Research Centre, where local and visiting mycologists can explore, gather and study fungi. We report *Psilocybe* spp. collected in 2010 in the area surrounding the Research Centre. We describe four species as new and discuss five species previously reported from Thailand.

Materials & methods

For microscopic study, thin hand sections of the basidiome, mainly in the pileus, including context and lamellae, were rehydrated with 96% alcohol and then mounted in 5% KOH and 1% Congo Red solutions (the latter added to tissues previously mounted in KOH). Basidiospore dimensions indicate length and width (face view) and thickness (side view). At least 25 measurements were taken for each microscopic feature. All specimens are deposited at Mae Fah Luang University herbarium (MFLU), Chiang Rai, Thailand, with duplicates in the Fungus Collection at XAL Herbarium, at Xalapa, Mexico.

Results

Five species of *Psilocybe* s.l. known from Thailand are discussed here (*P. cubensis*, *P. deconicoides*, *P. magnispora*, *P. samuiensis*, and *P. thailandensis*) and four new species are proposed.

Psilocybe cubensis (Earle) Singer, Sydowia 2: 37, 1948

≡ *Stropharia cubensis* Earle, Estudios Agronómicos, Cuba 1: 240, 1906

This is an important hallucinogenic mushroom with a pantropical distribution, although the records from Africa (Cullinan et al. 1945, Vedcourt & Trump 1969) are still under study to determine whether they represent *P. aquamarina* (Pegler) Guzmán (≡ *Stropharia aquamarina* Pegler), as discussed by Heim (1958, 1962), Pegler (1977), and Guzmán (1995). The Thai material (identified as *Stropharia cubensis*) studied by Heim, based on two collections (one by Apaiwongse, the second by Champagne) from the Bangkok region in 1957, agree well with the Mexican specimens studied by Heim. Recently Chamratpan (2003) and Klinhom et al. (2003) reported *P. cubensis* from Thailand as a "magic" and/or "soporific" mushroom. This coprophilous fungus, common on cattle and horse dung in meadows, probably grows also in Thailand on wild mammal dung. The African mentioned above was reported by Vedcourt & Trump (1969) on monkey dung, and Guzmán & Ramírez-Guillén (2010) reported the closely related *P. subcubensis* Guzmán on rhinoceros dung in Nepal. Nonetheless, Guzmán did not find *P. cubensis* nor *P. subcubensis* in meadows explored in Thailand in 2010, where he found only *Copelandia cyanescens* (Berk. & Broome) Singer and *Panaeolus* spp.

Psilocybe cubensis as shown in the key (below) has a well developed annulus, hyaline pleurocystidia, and thick walled basidiospores — (12–)13–15(–17) × 8–10(–11) × 7–9 µm — that are subhexagonal in face-view and subellipsoid in side-view (Guzmán 1983).

Deconica deconicoides (E. Horak, Guzmán & Desjardin) Guzmán, **comb. nov.**

MycoBank MB563261 FIGURES 1–3

≡ *Psilocybe deconicoides* E. Horak, Guzmán & Desjardin, Sydowia 61(1): 32, 2009

According to Horak et al. (2009) this is a non-bluing species, growing on rotting branches in Chiang Mai Prov. characterized by small mycenoid basidiomata with broadly adnate to decurrent lamellae. The thick-walled basidiospores — (5.5–)6–6.5 × (4.5–)5–5.5 × 3.5–4 µm — are rhomboid in face-view and ellipsoid in side-view. Basidia 17–23 × (5–)6.5–7 µm, 4-spored, hyaline, ventricose-clavate. Pleurocystidia absent. Cheilocystidia (14.5–)16–19(–24) × 5–6(–7) µm, polymorphic, ventricose, clavate, fusoid or sublageniform.

MATERIAL STUDIED — **THAILAND. CHIANG MAI PROV.**, Chiang Mai, Dui Suthep, 19 km marker, June 6, 2002, Horak 10156 (isotype XAL).

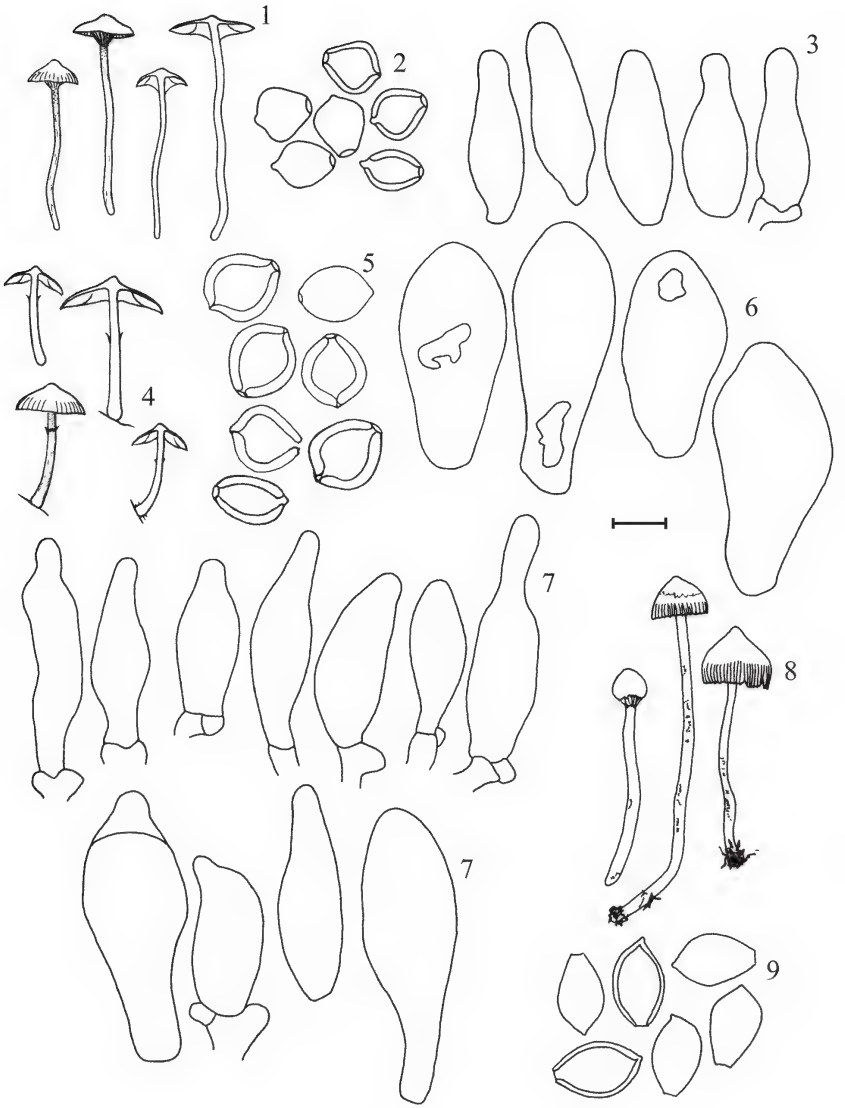
REMARKS: Moncalvo et al. (2002) showed two *Psilocybe* s.l. clades, one containing the bluing species and the other containing the non-bluing species. Redhead et al. (2007) proposed to conserve the name *Psilocybe* for the bluing clade and accommodate the non-bluing clade under *Deconica*. This proposal was supported by the Nomenclature Committee for Fungi (Norvell 2010) and accepted on July 31, 2011, by the 13th International Botanical Congress, Melbourne, necessitating the transfer of *P. deconicoides* to *Deconica*.

Psilocybe magnispora E. Horak, Guzmán & Desjardin, Sydowia 61: 25, 2009

FIGURES 4–7

This bluing species grows on old rotting elephant dung in a tropical montane forest and is only known from Pong Chang Province at Kha Yai (Horak et al. 2009). The basidiomata have a convex to obtusely papillate pileus (20–)30–45 mm diam, adnate lamellae, and stipe 35–60 × 2.5–4(–6) mm, with a submembranous annulus. Basidiospores are 7–8(–8.5) × (5.5–)6–7 × 5–5.5 µm, thick walled, and rhomboid in face-view. Pleurocystidia 21–28(–31) × (7–)9–13 µm, more or less common, ventricose-claviform or subfusoid, sometimes submucronate, hyaline, frequently with refringent incrustations, as chrysocystidia. Cheilocystidia (13–)16–26(–30) × 5–7(–10) µm, polymorphous, fusoid, ventricose or sublageniform, hyaline. The chrysocystidia and rhomboid spores place *P. magnispora* in sect. *Neocaledonicae* Guzmán.

MATERIAL STUDIED — **THAILAND, KHA YAI**, Pong Chang, Princess Trail, July 6, 2002, Horak 10171 (isotype XAL).



FIGURES 1–9. ***Deconica deconicoides*** (type) – 1. basidiomata; 2. basidiospores; 3. cheilocystidia. ***Psilocybe magnispora*** (type) – 4. basidiomata; 5. basidiospores; 6. pleurocystidia; (chrysocystidia); 7. cheilocystidia. ***P. samuiensis*** (type) – 8. basidiomata; 9. basidiospores. Scale bars: 1, 8 = 10 mm; 4 = 20 mm; 2–3, 5–7 = 5.5 µm; 9 = 10 µm.

Psilocybe samuiensis Guzmán, Bandala & J.W. Allen, Mycotaxon 46: 156, 1993

FIGURES 8–11

This species, known only from Thailand, grows on rich soil in meadows (Guzmán et al. 1993). It presents basidiomata like *P. mexicana* R. Heim, with pileus 7–15 mm diam., lamellae adnexed and stipe 40–65 × 1.5–2 mm, without annulus. Basidiospores 10–13 × 6–8 µm, rhomboid to subrhomboid in face-view, thick-walled. Pleurocystidia 16–20 × 5–6.5 µm, scattered, sublageniform, hyaline and cheilocystidia 18–28(–30) × (5–)5.5–7(–8) µm, sublageniform, sometimes branched.

Basidiospore morphology supports *P. samuiensis* within sect. *Mexicanae* Guzmán. Molecular analyses by Borovička et al. (2011) that show this species as separate from sects. *Cyanescens* and *Semilanceatae* support sect. *Mexicanae* as independent.

MATERIAL STUDIED — **THAILAND, SURAT THANI PROV.**, Koh Samui, Thailand, 2 km west of Ban Hua Thanon, August 8, 1991, Allen s.n. (holotype XAL).

Psilocybe thailandensis E. Horak, Guzmán & Desjardin, Sydowia 61: 30, 2009

FIGURES 12–15

This bluing species grows on rotten wood in a tropical montane forest (Horak et al. 2009). Pileus (4–)6–15 mm, conic, brown to pale brown, turning paler after drying, with veil remnants at the margin. Lamellae adnate. Stipe (10–)15–30 × 1–2 mm, subbulbous, with a fibrillose-membranaceous annulus, sometimes ephemeral. Basidiospores (4.5–)5–6(–6.5) × 4.5–5.5 × 3–4 µm, rhomboid in face-view, ellipsoid in side-view, thick–walled. Pleurocystidia of the chrysocystidia type with a refringent content, common, 16–25(–30) × (6.5–)7–9(–10), broadly clavate or vesiculose. Cheilocystidia (11–)20–30 × 3.5–6 µm, hyaline, sublageniform, ventricose-rostrate or irregularly slender subfusoid. Its chrysocystidia and rhomboid thick-walled spores place *P. thailandensis* in sect. *Neocaledonicae* with *P. magnispora*.

MATERIAL STUDIED — **THAILAND, CHIANG MAI PROV.**, Doi Suthep, Monthatharn Waterfall Trail, July 4, 2002, Horak 10128 (isotype XAL); trail to Huai Kok, Horak 10115 (paratype XAL).

Psilocybe thaiaerugineomaculans Guzmán, Karunarathna & Ram.-Guill., **sp. nov.**

MYCOBANK MB 561575 FIGURES 30–35

Differs from *Psilocybe aerugineomaculans* in producing pyriform yellowish brown pseudocystidia from the hymenophoral trama.

TYPE: Thailand, Chiang Mai Prov., Chiang Mai University Park, 18°48′N 98°53′E, elevation 300 m, July 27 2010, Karunarathna NTS-121 (**holotype** MFLU-10-0851; **isotype** XAL.

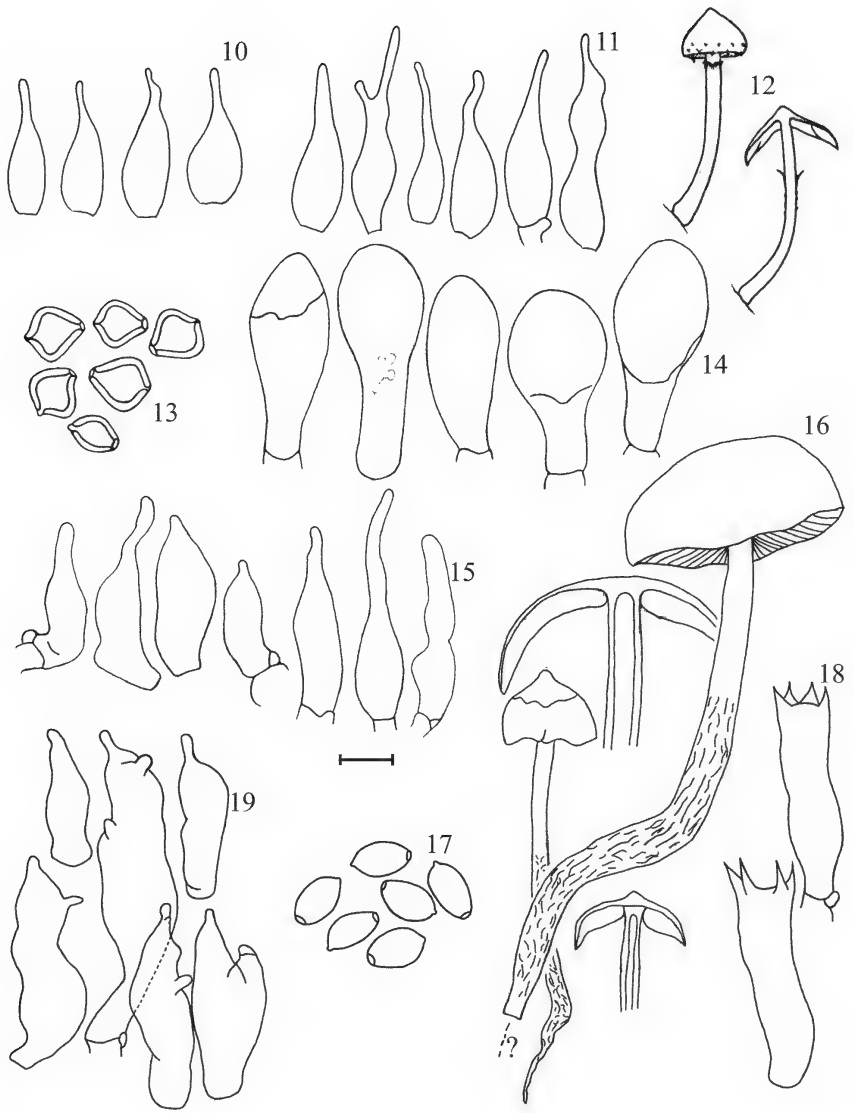
ETYMOLOGY: The epithet refers to Thailand and to the similar species *P. aerugineomaculans*.

Pileus 10–20 mm diam., conic to subumbonate, finally convex, smooth, glabrous, margin glabrous, striate when dry, hygrophanous, dark reddish brown to pale yellowish brown to whitish, bluing, finally blackish chocolate when dry. Lamellae broadly adnate to subadnexed, brownish violaceous to chocolate-brown, with white edges. Stipe 25–45 × (2–)3–4 mm, equal, subbulbous, smooth above, sub-floccose below, base fibrillose, pale yellowish brown to white or brownish black to reddish brown, hollow to stuffed, with whitish conspicuous mycelium at the base. Annulus membranous, white to off white, thick, persistent. Context white to pale rose, bluing upon exposure. Odor & taste not checked, in herbarium specimens none, but taste strongly pungent after some minutes. Spore print unknown.

Basidiospores 9–12 × 7–8(–9) × 5.5–7 µm, subrhomboid or subhexagonal in face-view, subellipsoid in side-view, thick-walled, wall 1–1.5 µ thick, yellowish brown, with a slightly truncate germ pore at the apex and a short, acute apiculus at the base. Basidia 21–31(–36) × (6–)7–10 µm, 4-spored, hyaline, cylindric-vesiculose to subclaviform, the sterigmata up to 6 µm long. Pleurocystidia (11–)14–22(–24) × 5–11(–13) µm, hyaline, thin-walled, more or less common, ventricose-mucronate to sub-rostrate or fusiform-globose or obovate. Pseudocystidia (25–)26–33(–35) × 11–16 µm, pyriform, content oily, yellowish brown, growing from the hymenophoral trama. Cheilocystidia 15–20 × (5–)6–8(–10) µm, hyaline, ventricose-mucronate to sub-rostrate or sublageniform, some fusiform or sub-ovate, rarely irregularly branched. Subhymenium subcellular, hyaline to yellowish pale, the elements 2–9 µm wide, thick-walled. hymenophoral trama regular, with hyphae 2–23 µm wide, hyaline, rarely pale yellowish, thin- to thick-walled. Pileipellis an ixocutis, 21–30 µm thick, with hyaline hyphae, 2–6 µm wide. Context with hyaline or some yellowish hyphae, 2–12 µm wide. Clamp connections present.

Habit, habitat and distribution — Solitary or gregarious at 300 m on soil with rotting cow dung, in an open grassland below the shadow of trees. Known only from the type locality.

Discussion. Possession of a stipe annulus on the stipe and thick-walled subrhomboid spores place *P. thaiaerugineomaculans* in sect. *Stuntzae* Guzmán. The pseudocystidia easily separate this species from all others in the section, mainly with the similar *P. aerugineomaculans* (Höhn.) Singer & A.H. Sm. from Java. That species, which has an ixocutis ≤ 10 µm thick and basidiospores 8.5–10.5 × 7–8 × 5.5–6.5 µm, lacks pseudocystidia and its cheilocystidia are 18–22 × 6–8 µm (Singer & Smith 1958). Another similar species, also from Java, is *P. subaeruginascens* Höhn., grows on dung, has 25–33 × 9–12 µm pleurocystidia and 20–33 × 6–9 µm cheilocystidia, lacks pseudocystidia, and its pileipellis is not gelatinous (Singer & Smith 1958). Guzmán (1983) referred



FIGURES 10–19. ***Psilocybe samuiensis*** (type) – 10. pleurocystidia; 11. cheilocystidia. ***P. thailandensis*** (type) – 12. basidiomata; 13. basidiospores; 14. pleurocystidia (chrysocystidia); 15. cheilocystidia. ***P. thaizapoteca*** (type) – 16. basidiomata; 17. basidiospores; 18. basidia; 19. pleurocystidia. Scale bars: 12, 16 = 10 mm; 10–11 = 8 µm; 13–15, 17–19 = 5.5 µm.

both Höhnel species to *P. subaeruginascens* but later (Guzmán 1995) separated them following Singer & Smith (1958). Recently Guzmán & Yang (2010) described *P. taiwanensis* Zhu L. Yang & Guzmán from Taiwan, also in the sect. *Stuntzae*, with conic to subumbonate pilei, (5.5–)6–7 × (3.5–)4–4.5 × 3.5–4 µm basidiospores, pileipellis with 8–10 × 4–5.5 µm cystidioid elements, and lacking pseudocystidia. We conclude that *P. thaiaerugineomaculans* is a well-defined Asiatic species independent from *P. aerugineomaculans*, *P. subaeruginascens*, and *P. taiwanensis*. A final note: Guzmán would refer the unidentified annulate *Psilocybe* from northern Thailand depicted in Stamets (1996: 171, 4th picture at bottom of color plate) to *P. thaiaerugineomaculans* based on form and color of the basidiomata, which agree well with the species here described.

Psilocybe thaicordispora Guzmán, Ram.-Guill. & Karunarathna, **sp. nov.**

MycoBank MB 561576 Figures 21–29

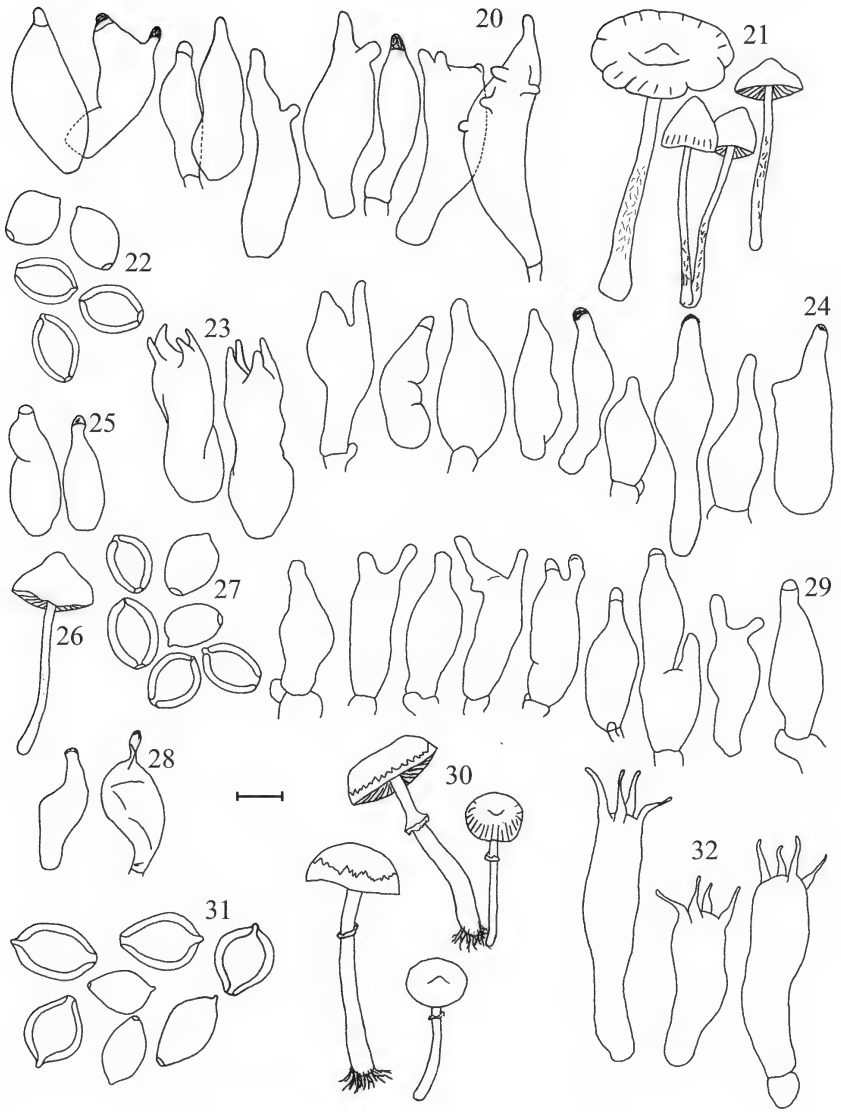
Differs from *Psilocybe thaicordispora* in narrower longer branched cheilocystidia and presence of pleurocystidia.

Type: Thailand, Chiang Mai Prov., approximately 100 km from Chiang Mai, near Huai Nam Dang National Park, (19°16′N 98°16′E), elevation 1500 m, rainforest dominated by *Pinus kesiya*, July 12, 2010; Guzmán 38349 (**holotype** MFLU-10-0804, **isotype** XAL).

Etymology: The epithet refers to Thailand and to the sect. *Cordisporae*.

Pileus 10–30 mm diam., conic to subcampanulate, finally convex or subconcave-umbonate, sometimes irregularly lobulate, sub-hygrophanous, yellowish brown to brownish orange, smooth, glabrous, without veil remnants, cuticle not separable. Lamellae subadnexed, yellowish pale to dark brown-violaceous, with whitish edges. Stipe 35–50 × 1–4 mm, uniform or subbulbous, smooth above to subfloccose toward the base or the latter is smooth, whitish above to reddish brown or reddish black. Veil poor development. Context white and thick below pileus, yellowish and fibrous in stipe. The entire basidiome intensely bluing and becoming blackish. Dried specimens are black-chocolate. Odor and taste slightly farinaceous. Spore print unknown.

Basidiospores (6.5–)7–8(–9) × (4.5–)5–5.5(–6.5) × 4.5–5 µm, subrhomboid or ovoid in face-view, subellipsoid in side-view, thick-walled, wall 1–1.5 µm thick, yellowish brown, with a truncate germ pore at the apex and a short acute apiculus at the base. Basidia 20–25 × 6–7 µm, 4-spored, hyaline, ventricose-subcylindric or subclaviform, with a middle constriction. Pleurocystidia 12–18(–24) × 3.5–7 µm, rare or more or less common, hyaline, thin-walled, ventricose, mucronate to rostrate. Cheilocystidia 12–20(–28) × (4.5–)5–7 (–8) µm, hyaline, thin-walled, polymorphous, ventricose, mucronate or rostrate, sometimes with a long cylindric base, apex frequently and irregularly bifurcate or branched. Subhymenium subcellular, with hyaline elements to incrusted by yellowish brown pigment, also with blue-green pigment. hymenophoral



FIGURES 20–32. ***Psilocybe thaizapoteca*** (type) – 20. cheilocystidia. ***P. thaicordispora*** (21–25 = type; 26–29 = Guzmán 38346) – 21. basidiomata; 22. basidiospores; 23. basidia; 24. cheilocystidia; 25. pleurocystidia; 26. basidiomata; 27. basidiospores; 28. pleurocystidia; 29. cheilocystidia. ***P. thaiaerugineomaculans*** (type) – 30. basidiomata; 31. basidiospores; 32. basidia. Scale bars: 21, 26, 30 = 10 mm; 20, 22–25, 27–29, 31–32 = 5.5 µm.

Trama regular, with hyphae (2–)3–20(–25) µm wide, thin- or thick-walled, up to 0.8 µm thick, branched, hyaline. Pileipellis an ixocutis, 12–20 µm thick, with hyphae, 2–5 µm wide, hyaline, branched. Context subcellular, hyaline to pale brownish through the pileipellis, with hyphae 3–9(–23) µm wide, hyaline, some branched. Clamp connections present.

Habit, habitat and distribution — Solitary or gregarious, on grassy soil, below the shadow of trees, in an open subtropical forest, at 1500 m elevation. Known only from the type locality.

Additional material studied — **THAILAND, Chiang Mai Prov.**, approximately 100 km from Chiang Mai, near Huai Nam Dang National Park, (19°16′N 98°16′E), elevation 1500 m, rainforest dominated by *Pinus kesiya*, July 12, 2010; Guzmán 38346 (MFLU-10-0801; XAL).

Discussion. *Psilocybe thaicordispora* belongs to sect. *Cordisporae* Guzmán because of its small spores, which are ovoid or subrhomboid in face-view, thick-walled and have a broad germ pore, as well as in its tropical habitat. It is somewhat similar to *P. papuana* Guzmán & E. Horak known only from Papua New Guinea, and also has polymorphous cheilocystidia, but these are wider, shorter and un-branched, (6.5–)10–18 × 6.5–13 µm, and apparently has no pleurocystidia (Guzmán & Horak 1978). Another similar species is *P. dumontii* Singer ex Guzmán known only from Panama, but this species has un-branched cheilocystidia which are not so variable in form, 10–18(–26) × 3–6(–8) µm, hyaline to somewhat brownish (Guzmán 1978, Guzmán & Piepenbring 2011).

Psilocybe thaiduplicatocystidiata Guzmán, Karunarathna & Ram.-Guill., **sp. nov.**

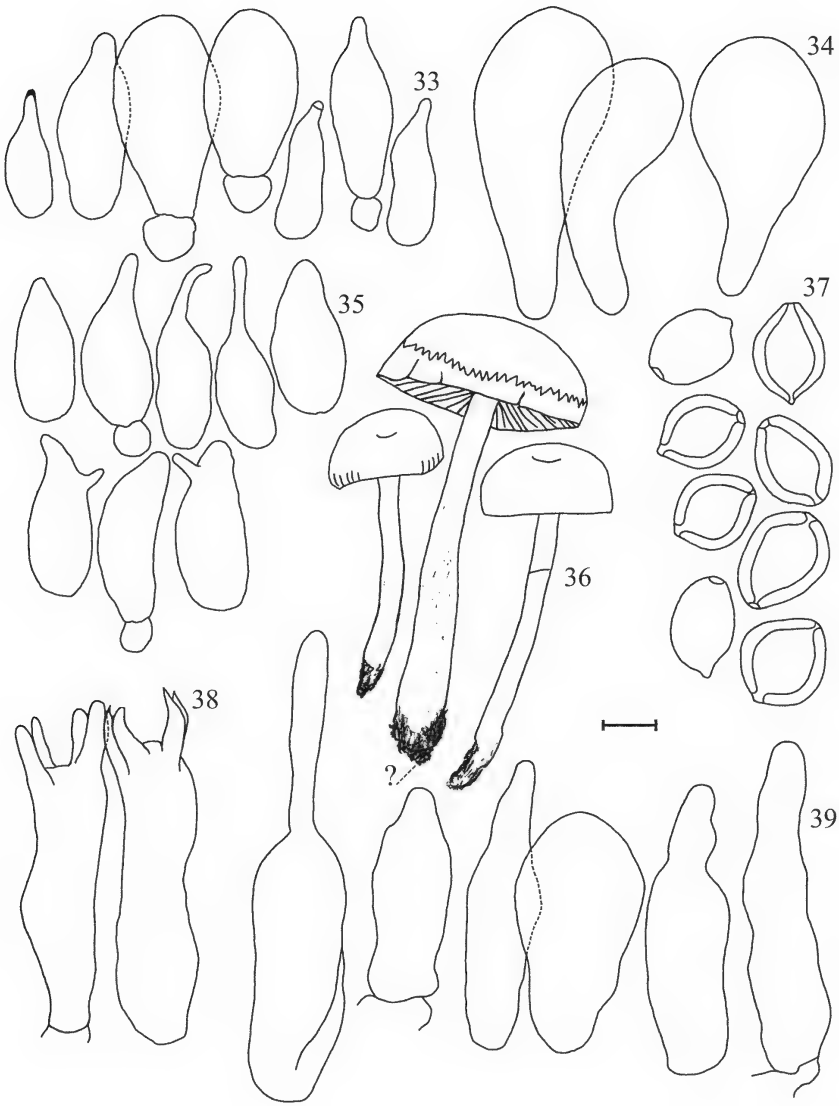
MycoBank MB 561577 Figures 36–42

Differs from *Psilocybe caerulescens* in the possession of pleurocystidia, polymorphic cheilocystidia, and longer basidiospores.

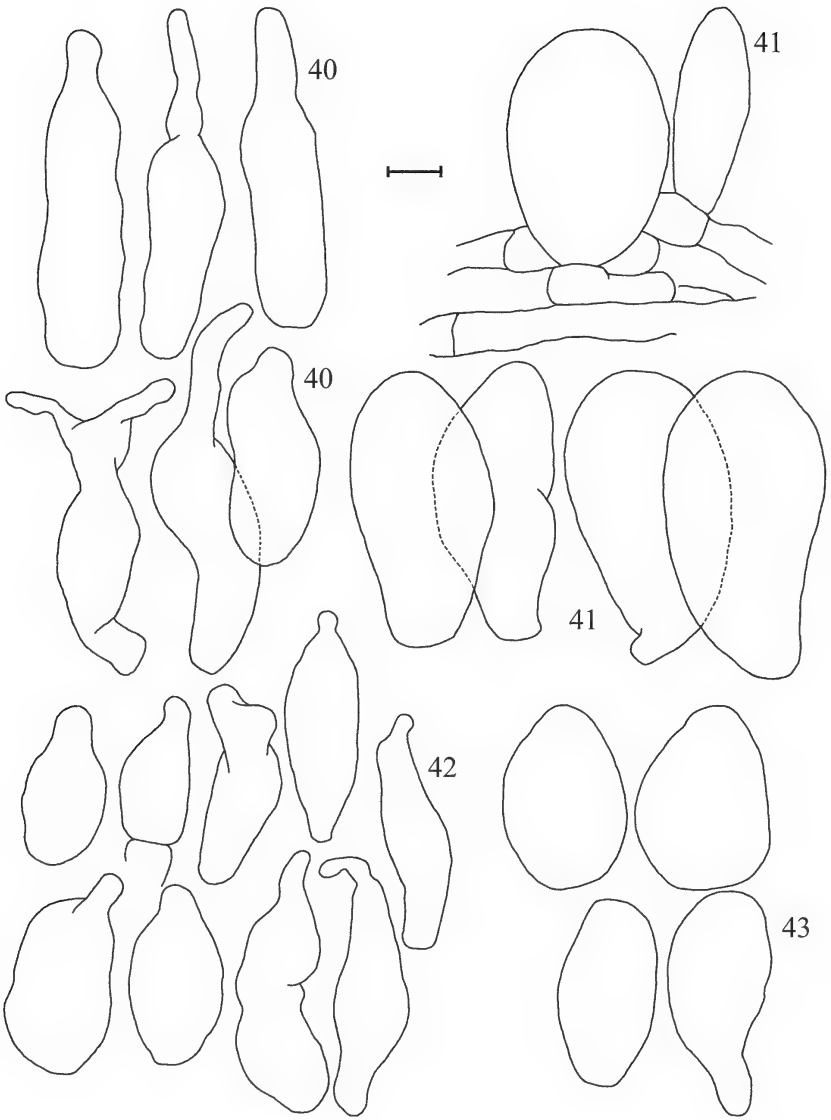
Type: Thailand, Chiang Mai Prov., Chiang Mai University Park, 18°48′N 98°53′E, elevation 300 m, July 27, 2010, SC Karunarathna NTS-120 (**holotype** MFLU-10-0850, **isotype** XAL).

Etymology: The epithet refers to Thailand and the dimorphic pleurocystidia and cheilocystidia.

Pileus 20–40 mm diam., convex to subumbonate or subumbilicate, glabrous, smooth but margin slightly striate, also sometimes sub-lobulate, sub-hygrophanous, pale brown or brownish rose to brownish dull orange at the center, pale yellowish brown when dry, bluing to becoming irregularly blackish chocolate. Lamellae adnate to seceding, black-violaceous to blackish chocolate, with white edges. Stipe 45–75 × 4–6 mm, equal, subbulbous, probably with a pseudorhiza (not gathered), surface white to pale to dark reddish brown, or pale grayish brown when dry, smooth above to sub-floccose below, hollow to stuffed. Context white to pale rose, bluing. Odor and taste not checked



FIGURES 33–39. ***Psilocybe thaiaerugineomaculans*** (type) – 33. pleurocystidia type A; 34. pleurocystidia type B; 35. cheilocystidia. ***P. thaiduplicatocystidiata*** (type) – 36. basidiomata; 37. basidiospores; 38. basidia; 39. pleurocystidia type A. Scale bars: 36 = 10 mm; 33–35, 37–39 = 5.5 µm.



FIGURES 40–43. ***Psilocybe thaiduplicatocystidiata*** (type) – 40. pleurocystidia type A; 41. pleurocystidia type B; 42. cheilocystidia type A; 43. cheilocystidia type B. Scale bar = 5.5 µm.

when fresh, without any odor or taste in herbarium specimens, but the taste after some minutes is strongly pungent. SPORE PRINT unknown.

Basidiospores 9–10(–11) × 6.5–7(–8) × 5.5–6.5 µm, subrhomboid or obscurely subovoid in face-view, subellipsoid in side-view, thick-walled, wall 1–1.5 µm thick, yellowish brown, with a truncate germ pore at the apex and a short acute apiculus at the base. Basidia 25–36 × 7–8(–9) µm, 4-spored, hyaline, ventricose-subcylindric or subclaviform, with a middle constriction, sterigmata up to 12 µm long. Pleurocystidia polymorphous, arising from the subhymenium, of two types: (A) 20–37(–44) × 7–9.5 µm, hyaline, ventricose-subcylindric, short rostrate or with a long narrow and cylindric neck, sometimes branched; (B) 22–29 × 10–15 µm, as pseudocystidia, hyaline or yellowish pale, subglobose, subfusiform or ovoid, thin-walled. Cheilocystidia polymorphous, of two types: (A) 16–22(–27) × 6.5–9.5 µm, fusiform, ventricose, rostrate, regular or irregularly, apex short or long, sometimes lobulate; (B) 18–24 × 9.5–13 µm, rare, as pseudocystidia, grayish, arising from the hymenophoral trama. Subhymenium thick, hyaline, subcellular, globose elements 5.5–13 (–20) µm wide. hymenophoral Trama regular, with hyaline to yellowish brown hyphae, 2–16 µm wide. Pileipellis subgelatinous, with hyaline hyphae, 2–4 µm wide. Context with hyaline to yellowish hyphae, 3.5–34 µm wide, thin-walled. Clamp connections present.

Habit, habitat and distribution — Gregarious, on soil with rotting dung, in an open grassland, at 300 m elevation. Known only from the type locality.

Discussion. *Psilocybe thaiduplicatocystidiata* with its small, subrhomboid, thick-walled spores is a special member of the sect. *Cordisporae* as it has two types of both pleurocystidia and cheilocystidia, one as pseudocystidia, a feature not previously observed on any species of sect. *Cordisporae* (Guzmán 1983, 1995). This species may also possess a pseudorhiza, but this was lost during picking. The basidiome is similar to *P. caerulescens* Murrill, but that species lacks pleurocystidia, the cheilocystidia are not polymorphous and its basidiospores are (6–)6.5–8(–8.5) µm long. *P. thaiduplicatocystidiata* is also similar to *P. wrightii* Guzmán, but the latter has simple pleurocystidia and cheilocystidia, and also smaller spores. These two similar species are only known from the neotropics.

Psilocybe thaizapoteca Guzmán, Karunarathna & Ram.-Guill., **sp. nov.**

MycoBank MB 561578 Figures 16–20

Differs from *Psilocybe angustipleurocystidiata* in a thick subgelatinous pilleipellis and presence of branched, lobed pleurocystidia.

Type: Thailand, Chiang Mai Prov., close to Huai Nam Dang National Park (19°16′N 98°16′E), elevation 1500 m, rainforest dominated by *Pinus kesiya*, July 12, 2010, Guzmán 38342 (**holotype** MFLU-10-0797, **isotype** XAL).

Etymology: The epithet refers to Thailand and similarity of the fungus to the *P. zapotecorum*-group.

Pileus (20–)30–50 mm diam., conic-umbonate to campanulate, finally convex to plano-convex, sometimes irregularly lobulate, glabrous overall, smooth, lubricous to soon dry, cuticle not separable, sub-hygrophanous, brownish red with chocolate tints, at the margin without veil remnants. Lamellae adnexed or short sinuate, brownish chocolate, edges whitish. Stipe 70–120 × 4–8 mm, uniform but narrow toward the base, hollow, sub-fleshy to fibrous, whitish above, brownish red or brownish purple to whitish gray or dark vinaceous at the base. Pseudorhiza as a long irregular whitish cordon. Veil poorly developed as fibrillar remnants. Context white to whitish, fleshy, bluing to blackish. Odor and taste slightly farinaceous at first, but the taste also a little pungent in fresh specimens. Spore print unknown.

Basidiospores 6–6.5(–7) × (3–)3.5–4(–4.5) × 3–3.5 µm, subellipsoid or obscurely subrhomboid in face-view, subellipsoid on side-view, thin-walled, wall 0.5–0.8 µm thick, pale yellowish brown, with a truncate germ pore at the apex and a short acute apiculus at the base. Basidia 19–25 × (4.5–)5–5.5(–6) µm, 4-spored, hyaline, ventricose, subcylindric, or claviform with a middle constriction. Pleurocystidia 13–20(–30) × (3–)4–6(–7.5) µm, common, hyaline, subventricose or subcylindric regular or irregularly but acute mucronate, frequently irregularly branched or lobed. Apparently there are two kind of pleurocystidia, smaller and bigger, but these latter are very rare. Pseudocystidia absent. Cheilocystidia 15–22(–25) × 4–7(–8) µm, hyaline, subventricose, somewhat claviform, all of them mucronate, regular or irregularly, also irregularly lobulate or subbranched. Subhymenium subcellular, hyaline, with thin-walled, somewhat incrusted hyphae. hymenophoral Trama regular, with 3–30 µm wide, hyaline to yellowish or pale brownish hyphae, some of them swollen. Pileipellis as thin layer, not gelatinous, with hyaline hyphae, 1–2(–5) µm wide, without pileocystidia. Context like the hymenophoral trama hyphae. Clamp connections present.

Habit, habitat and distribution — Gregarious, on muddy soil with few herbs, mosses and grasses, in a tropical-subtropical vegetation, with Dipterocarpaceae trees and some *Pinus*. Known only from the type locality.

Discussion. Its thin-walled, subellipsoid spores place *P. thaizapoteca* in sect. *Zapotecorum* Guzmán, reported the first time from Thailand. *Psilocybe angustipleurocystidiata* Guzmán (known only from Colombia and Mexico), which also lacks pseudocystidia, differs in its thick (10–15(–20) µm) subgelatinous pileipellis and pleurocystidia that are neither branched nor lobed. *Psilocybe aucklandiae* Guzmán et al. (known only from New Zealand) is differentiated by the subgelatinous pileipellis and unbranched cheilocystidia and pleurocystidia. *Psilocybe kumaenorum* R. Heim (known only from Papua New Guinea) is also similar to *P. thaizapoteca* but is distinguished by small basidiomata (pileus 5–7 mm diam, stipe 15–30 × 1–2 mm) and non-lobed cheilocystidia (Heim 1966).

Key to species of *Psilocybe* s.l. reported from Thailand

(bold = new species described in the present paper)

1a. Non-bluing species (excluded from *Psilocybe* s.s.) ..... *Deconica deconicoides*
1b. Bluing species 2

2a. Spores thin-walled, rhomboid or subellipsoid in face-view.
On muddy soil ..... ***P. thaizapoteca***
2b. Spores thick-walled, subrhomboid or subhexagonal in face-view.
Not on muddy soil 3

3a. Annulus present 4
3b. Annulus absent 7

4a. Spores (12–)13–15(–17) µm long, subhexagonal in face-view.
Chrysocystidia absent ..... *P. cubensis*
4b. Spores smaller, up to 12 µm long. Chrysocystidia present or absent. 5

5a. Chrysocystidia absent. Spores 9–12 µm long. Growing on litter
..... ***P. thaiaerugineomaculans***
5b. Chrysocystidia present 6

6a. Spores 7–8 µm long. On elephant dung ..... *P. magnispora*
6b. Spores (4.5–)5–6(–6.5) µm long. On rotten wood ..... *P. thailandensis*

7a. Pleurocystidia and cheilocystidia of two types. Growing on soil
..... ***P. thaiduplicatocystidiata***
7b. Pleurocystidia and cheilocystidia of one type. On grassy soil 8

8a. Spores 10–13 µm long ..... *P. samuiensis*
8b. Spores (6.5–)7–8(–9) µm long ..... ***P. thaicordispora***

Final considerations

It is interesting that four new species were found during such a short excursion time (five days) in Thailand, while none of the five species previously reported for the country were found. We suggest this demonstrates the complexity of mushroom fructification and at same time the richness of the mycota in the tropics. We confirmed that species of *Psilocybe* s.s. sects. *Cordisporae*, *Mexicanae, Stuntzae,* and *Zapotecorum* are tropical or subtropical. We also observed that bluing species have a greatly varied habitat, for example, litter, muddy soil, rotten wood or dung. Although the odor and taste of basidiomata are generally farinaceous for all bluing *Psilocybe* species, for Thai species odor and taste are either not distinctive or acidulous to only very slightly farinaceous, with the taste of dried material in all four new species (as well of fresh basidiomata in *P. thaizapoteca*) becoming strongly pungent after some minutes.

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A new *Corynesporella* species and two first records from China

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Abstract — A new species, *Corynesporella obclavata,* is described and illustrated from dead branches of *Pistacia chinensis* collected from tropical forests in Yunnan Province, China. A synopsis of *Corynesporella* species is provided. *Stephembruneria elegans* and *Diplococcium pulneyense* are recorded for the first time from China.

Key words — anamorphic fungi, taxonomy

Introduction

Numerous fungi on wood have been discovered in tropical and subtropical forests during recent research on the fungus flora of South China (Dai et al. 2009, Dai & Li 2010, Ma et al. 2010, 2011). In our continuing study of conidial fungi on dead wood, a hitherto undescribed species of *Corynesporella* and two new records were found. These three species are described, illustrated, and compared with similar species. The specimens are deposited in HSAUP (Herbarium of the Department of Plant Pathology, Shandong Agricultural University) and HMAS (Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences).

Taxonomy

Corynesporella obclavata L.G. Ma & X.G. Zhang, **sp. nov.** Fig. 1

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Conidiophora macronematica, ramosa, 230–635 µm longa, ad apicem 3.5–6.5 µm lata, ad basem 8.5–15 µm lata. Cellulae conidiogenae monotreticae, in conidiophoris incorporatae, terminales, 7.5–16 × 3.5–7.5 µm. Conidia solitaria, acrogena, obclavata, brunneae, cellula apicali pallide brunnea, laevibus, 33–55 × 6.5–11 µm, 5–7-pseudoseptata.

Type: China, Yunnan Province: the Forbidden Forest of Banna, on dead branches of *Pistacia chinensis* Bunge (*Anacardiaceae*), 17 Oct. 2008, L.G. Ma (**holotype**, HSAUP H0033; **isotype**, HMAS 146152).

Etymology: *obclavata*, in reference to the conidial shape.

Table 1. Comparison of *Corynesporella* species.

Species	Conidiophores		Conidiogenous cells	Conidia				
	size (µm)	branching	size (µm)	size (µm)	catenate	color	septation	shape
C. bhowaliensis	920–1120 × 10–14	yes	32–64 × <8	70–192 × 8–10	no	subhyaline to pale brown	15–32	vermiform, cylindrical or subcylindrical
C. helminthosporioides	95–900 × 8–20	yes	9–18 × 6.5–10	50–75 × 8.8–12.8	no	subhyaline	9–13	obclavate, occasionally rostrate
C. obclavata	230–635 × 8.5–15	yes	7.5–16 × 3.5–7.5	33–55 × 6.5–11.0	no	brown	5–7	obclavate
C. pinarensis	<350 × 7–14	no	10.5–20 × 4–5	25–75 × 3.5–7	yes	brown to olive brown	(1–)3(–5)	obclavate
C. simpliphora	150–600 × 6.5–8	no	–	18–62 × 6.5–10	yes	pale olive	1–7	obclavate
C. superioramifera	20–160 × 2–5	yes	8–22 × 4–4.5	5–90 × 2.5–9	yes	moderately black	0–5	cylindrical
C. urticae	786–1048 × 13–19	yes	–	98–270 × 7–12	yes	subhyaline to pale brown	5–29	subcylindrical or obclavate

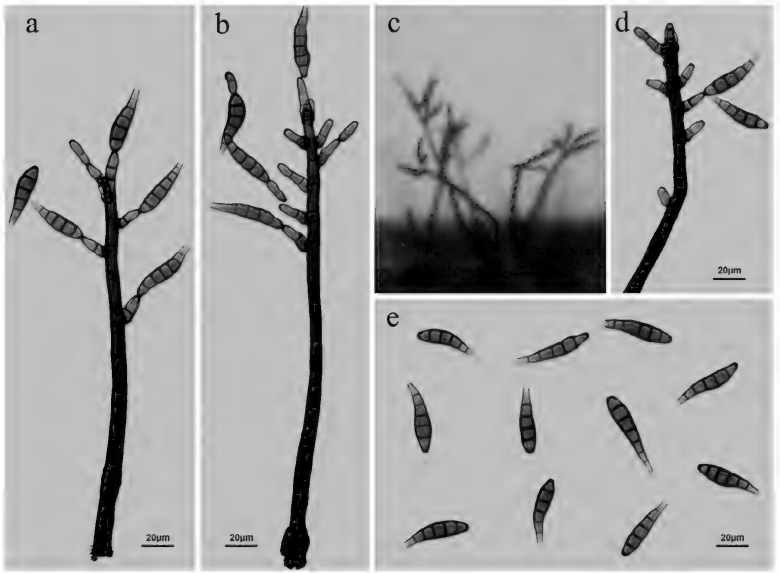

Fig. 1. *Corynesporella obclavata*. a, b. Branched conidiophores, conidiogenous cells and conidia. c. Conidiophores arising from wood. d. Conidiogenous cells. e. Conidia.

Colonies on natural substrate effuse, brown to dark brown. Mycelium partly immersed, partly superficial, composed of branched, septate, pale brown, smooth hyphae, 2–4 μm wide. Conidiophores macronematous, mononematous, erect, branched, straight or flexuous, cylindrical, brown, thick-walled, multiseptate, smooth, 230–635 μm long, 3.5–6.5 μm wide at the apex, 8.5–15 μm wide at the base. Conidiogenous cells monotretic, integrated, terminal, cylindrical, smooth, brown, thick-walled, 7.5–16 × 3.5–7.5 μm. Conidia solitary, acrogenous, obclavate, brown, apical cells pale brown, smooth, thick-walled, 5–7-distoseptate, 33–55 × 6.5–11 μm.

All seven *Corynesporella* species are presented in Table 1. In conidial shape, *C. obclavata* is most similar to *C. helminthosporioides* (Holubová-Jechová 1987), which can be easily separated by its larger (50–75 × 8.8–12.8 μm) subhyaline conidia with more septa (9–13-distosepta).

Stephembruneria elegans R.F. Castañeda, Fungi Cubenses III: 14, 1988. Fig. 2

Colonies on natural substrate effuse, brown. Mycelium partly superficial, partly immersed, composed of branched, septate, smooth, pale brown to brown hyphae, 1–2 μm wide. Conidiophores macronematous mononematous, unbranched, erect, straight to slightly flexuous, smooth, dark brown, cylindrical, thick-walled, paler and narrower at the apex, multiseptate, 170–290 μm long, 5–11.5 μm wide. Conidiogenous cells monophialidic, cylindrical, integrated,

terminal, percurrent, smooth, pale brown to subhyaline, with a minute collar at the apex, 2.5–3.3 um wide. Conidia solitary, phialidic, navicular to fusiform, rounded at the apex, with a narrow hilum at the base, smooth, 6–7-euseptate, simple, end cells subhyaline, central cells dark brown, 26.5–33.5 μm long, 10–11.5 μm wide.

Specimen examined: **CHINA, Hainan Province**: the National Natural Reserve of Bawangling, on dead branches of *Ficus benjamina* L. (*Moraceae*), 23 Nov. 2008, L.G. Ma (HSAUP H0514, HMAS 146149).

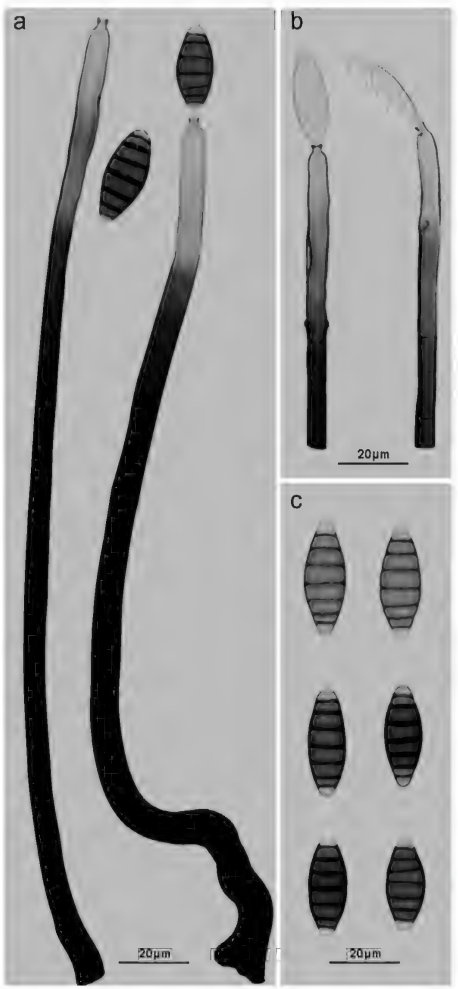

Fig. 2. *Stephembruneria elegans*. a. Conidiophores, conidiogenous cells and conidia. b. Conidiogenous cells with immature conidia. c. Conidia.

The genus *Stephembruneria* was erected by Castañeda Ruíz (1988) to accommodate a single species, *S. elegans*. Currently *Stephembruneria* remains monotypic and is characterized by macronematous, mononematous conidiophores, monophialidic, terminal, integrated, conidiogenous cells that narrow to a slightly cupulate collarette at the apex, and solitary, euseptate, navicular to fusiform, versicolored conidia. The type material differs from our material by its larger conidia (30–43 × 10–13 µm) and longer conidiophores (250–350 µm). This is the first record from China.

Fig. 3. *Diplococcium pulneyense*.
a. Branched conidiophores, conidiogenous cells and conidia. b. Conidia.

Diplococcium pulneyense Subram. & Sekar, Kavaka 15: 91, 1989. Fig. 3

Colonies on natural substrate effuse, dark brown, hairy. Mycelium partly superficial, partly immersed, composed of branched, septate, pale brown to brown, smooth hyphae, 2–3 µm wide. Conidiophores macronematous, mononematous, branched, branches often short, erect, cylindrical, straight

or flexuous, smooth, brown, thick-walled, 6–12-septate, 110–265 µm long, 8.5–10 µm wide at the base, 5–7.5 µm wide at the apex. Conidiogenous cells integrated, polytretic, terminal and intercalary, cylindrical, brown, smooth, thick-walled. Conidia in acropleurogenous chains, up to 7 in number, ellipsoidal, subcylindrical, 1–(2)-euseptate, rounded at both ends, concolored, brown, smooth, thick-walled, 10–18.5 × 6–7.5 µm.

Specimen examined: **China, Guangdong Province**: the National Forest Park of Shimen, on dead branches of *Bauhinia blakeana* Dunn (*Caesalpiniaceae*), 20 Oct. 2010, L.G. Ma (HSAUP H1787, HMAS 146150).

Diplococcium pulneyense is most similar to *D. spicatum* in conidial shape and septation (Grove 1885), but *D. spicatum* can be distinguished by its smaller conidia (6–9 × 3–4 µm). The type material differs from the Chinese specimen by its longer conidia (12–30 µm) and narrower conidiophores (3–5.5 µm). This is a new record from China.

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Queletia mirabilis, a rare gasteroid species in Gran Canaria, Spain

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Abstract — This paper provides the first report of a rare gasteroid taxon, *Queletia mirabilis,* from the Canary Islands, accompanied by color photographs of its macro- and microscopic characteristics and scanning electron micrographs of spore ornamentation and capillitium. A comparison with other similar species of *Queletia* and *Schizostoma* is also given.

Key words — *Agaricaceae, Basidiomycota,* taxonomy

Introduction

Queletia mirabilis is a very rare species. According to Lloyd (1904) and Azema (1990), it was first collected by the mycologist, Perdrizet of Vaudancourt (Oise), in Port de Sochaux (France) around 1868 and sent to Lucien Skeleton, a doctor in Hérimoncourt (Doubs), who eventually passed it to Fries, who classified and named it. This species has very few citations and is considered by some mycologists as "an alien species." *Queletia mirabilis* is known only from France (Dumée & Maire 1913, Mornand 1989, Azema 1990), England (Ramsbottom 1953, Pegler et al. 1995), Italy (Cetto 1980, Sarasini 2005), and USA (White 1901, Lloyd 1904, Coker & Couch 1928). The species has also been described from different authors' herbarium material such as Fischer (1900), Lloyd (1903), Pilát (1955), Moravec (1958) and Jülich (1983). For the first time below, we describe *Q. mirabilis* from the island of Gran Canaria, Canary Islands (Spain), in the Macaronesian region and provide SEM illustrations of its spore and capillitial ultrastructure.

Materials & methods

The studied specimens have been deposited in the herbaria of AH (University of Alcalá, Spain) and the Botanical Garden "Viera y Clavijo" (Gran Canaria, Spain) and

the personal herbarium of M.A. Ribes (MAR). Glebal tissue was mounted in Hoyer's medium and studied with a Nikon microscope. Spore measurements were made using an oil immersion objective. The scanning electron microscope (SEM) photographs were made according to Moreno et al. (1995).

Taxonomy

Queletia mirabilis Fr., Öfvers. Förhandl. K. Svenska Vetensk.-Akad. 28: 171 (1872)
FIGS. 1–12

BASIDIOMATA scattered to gregarious, stipitate. PILEUS globose to subglobose, 3–4 cm in diam., 1.5–2 cm high. PERIDIUM mostly missing, only near the base in contact with foot, composed of a membrane, 3–5 mm thick, with smooth, whitish outer surface; rough inner surface, irregular, brown to ochre, with abundant remains of capillitium and spores. GLEBA ocher, abundant, with filaments of whitish capillitium. DEHISCENCE apical, irregular, without formation of a stoma or defined structure. STIPE 0.7–1.2 × 6–10 cm, cylindrical, curved, fading towards base, not twisted, with a strongly folded surface and appearing torn, ochre and brown, woody. COLUMELLA absent.

BASIDIOSPORES yellow-ochraceous, globose to subglobose, (4.9–)5.4–6.6 (–7.8) × (4.4–)4.9–6.2(–6.8) µm, excluding ornamentation; Q = 1.0–1.2(–1.4), N = 94, I = 6.0 × 5.5 µm, Q = 1.1, with a prominent hilar appendix, strongly verrucose, with verrucae grouped into conical structures (0.8–)1.1–1.5(–1.7) µm, N = 52, I = 1.3 µm high. BASIDIA not seen. CAPILLITIUM abundant, cylindrical, twisted, wavy-helical without fibulae, ocher, thick-walled, poorly branched and rarely septate, that breaks easily, probably as a result of handling (4.4–)5.6–8.9(–10.3) µm, I = 7.1 µm wide.

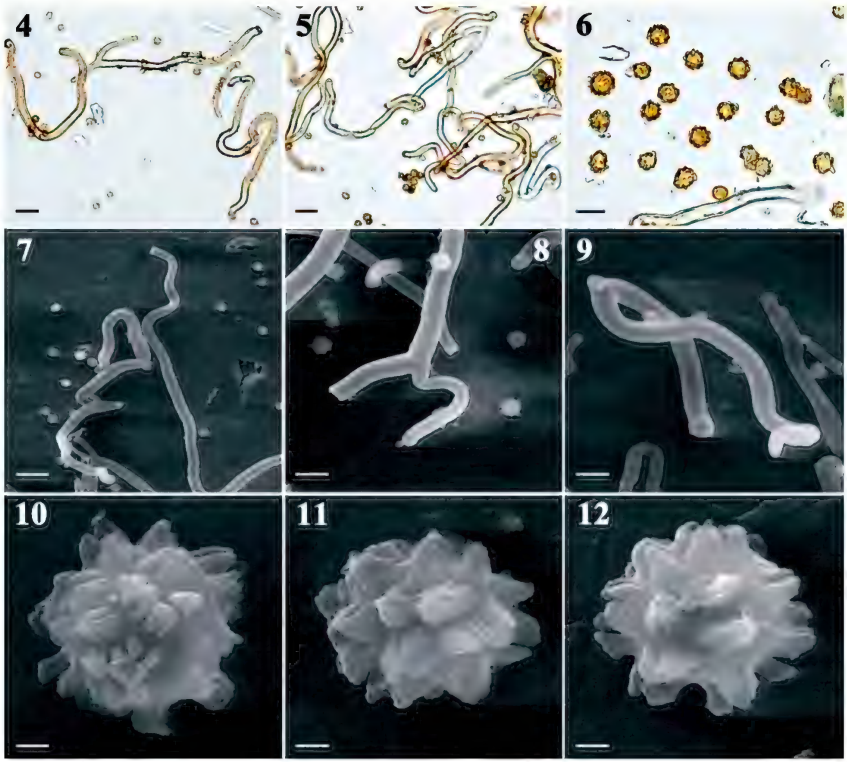
SEM revealed spore ornamentation consisting of long rods that join together to form pyramidal structures. This type of spore ornamentation matches *Q. mirabilis* from France deposited at PC (Jülich 1983).

MATERIAL EXAMINED: **SPAIN**: **CANARY ISLANDS**, GRAN CANARIA, Tejeda, close to the "Presa de la Data" dam, (27°55'52.35"N, 15°39'17.32"W) in sandy soil in grass near dam, flooded all year round with seasonal variations, surrounding vegetation scattered *Pinus canariensis* C. Sm. ex DC., a thicket of legumes [*Teline microphylla* (DC.) PE Gibbs & Dingwall and *Chamaecytisus proliferus* ssp. *meridionalis* Acebes] and "tabaibas" bitter (*Euphorbia regis-juba* Webb & Berthel.), with "verodes" (*Kleinia neriifolia* Haw.), 16-X-2010, leg. M. Cardona Sosa, det. Gabriel Moreno, AH 39331, MAR 161010-25, duplicate in Botanical Garden "Viera y Clavijo" SMGC (Soc. Mycol. Gran Canaria) 11043. **ITALY**: **FRIULI-VENEZIA GIULIA**, UDINE, Gorizia, Rindenabfällen von auf bei Laubholz Torviscosa, with substrate temperature 38-40° C, 25-VIII-1980, leg. Tarcisio Figar misit B. Cetto, det. Tarcisio Figar, Hanns Kreisel ex Herbarium, duplicate in AH 25235.

OTHER MATERIAL STUDIED: ***Queletia andina*** —**ARGENTINA**, **MENDOZA**, San Martín, leg. J. Semper, 15-VII-1950, Ruiz Leal 16204 (holotype, BAFC 28711;, isotype, AH 39332). ***Queletia mundkurii*** —**PAKISTAN**, **PUNJAB**, Ladhar, on ground, 18-VIII-1947, LAH, duplicate in AH 39333.

Fig. 1–3: *Queletia mirabilis* (AH 39331).
1. Fruiting bodies. 2–3. Details of gleba (2) and capillitial remnants (3). Scale bars = 0.5 cm.

Comments: Although *Queletia mirabilis* is a rare species, it is easily distinguished by its irregular peridium dehiscence, absence of columella, filamentous, helical-wavy, winding capillitium, and strongly ornamented basidiospores. Development of the fruiting bodies, details of peridium dehiscence, basidia



Figs 4–12: *Queletia mirabilis* (AH 39331).
4-6. Details of capillitium (LM, 4–5) and spores (LM, 6) in distilled water).
7–12. SEM photos: 7–9. Capillitium and branches; 10–12. Spore ornamentation.
Scale bars: 4–6 and 8–9 = 10 µm, 7 = 20 µm, 10–12 = 1 µm.

formation, and development of the spores have been described in detail by Dumée & Maire (1913).

De Toni's (1888: 65) description of *Q. mirabilis* differs from those of other mycologists because he described comparatively larger basidiospores (10–15 µm diam.). In general, the basidiospores are smaller (7.5–9.2 µm including ornamentation) than given by De Toni (1888). The size of the fruiting bodies also differs. According to De Toni (1888), the stipe is 1–2 cm long with a base up to 4.5 cm wide and apex 2.5 cm broad.

This gasteroid fungus is usually found fruiting on bark and wood debris (Cetto 1980, Azéma 1990, Pegler et al. 1995, Sarasini 2005). It rarely produces fruiting bodies on the ground after rains (De Toni 1888), an important character. The sample from the Canary Islands grew in sandy soil that remains waterlogged all year round but with seasonal variations.

The genera *Queletia* Fr. and *Schizostoma* Ehrenb. ex Lév., are closely related by the wavy-helical, aseptate or rarely septate, shortly branched capillitium. However, *Schizostoma* differs in possessing smooth basidiospores and a peridium that becomes irregular and stellate at maturity.

The only other species currently accepted in this genus is *Queletia andina* J.E. Wright, which is characterized by its filamentous, branched capillitium with abundant septa (*Tulostoma* type) that do not break under the pressure and smooth basidiospores. The capillitium of *Schizostoma mundkurii* (S. Ahmad) Long & Stouffer [≡ *Queletia mundkurii* S. Ahmad] is similar to that of *Q. mirabilis* but presence of smooth spores and other generic characters of the former differentiate these two species.

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New species and a new record of *Solicorynespora* from southern China

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Abstract — Four species of *Solicorynespora* were collected on dead branches from tropical or subtropical forests in southern China. The new species *S. ligustri* on *Ligustrum quihoui*, *S. machili* on *Machilus chinensis*, and *S. cryptocaryae* on *Cryptocarya chinensis* are described, illustrated, and compared with closely related taxa. *Solicorynespora pseudolmediae* is recorded for the first time from China.

Key words — anamorphic fungi, taxonomy

Introduction

Solicorynespora R.F. Castañeda & W.B. Kendr., erected by Castañeda & Kendrick (1990) with *S. zapatensis* R.F. Castañeda & W.B. Kendr. as the type species, was mainly characterized by conspicuous, single, determinate or percurrently extending conidiophores and integrated, terminal, monotretic conidiogenous cells with solitary, obclavate or pyriform, euseptate phragmoconidia. These characters separate *Solicorynespora* from similar genera including *Corynespora* Güssow (Güssow 1906), *Corynesporella* Munjal & H.S. Gill (Munjal & Gill 1961), *Hemicorynespora* M.B. Ellis (Ellis 1972), and *Corynesporopsis* P.M. Kirk (Kirk 1981). Keys to *Solicorynespora* species have been given by Castañeda et al. (2004) and Ma et al. (2011b), based mainly on conidial morphology. Until now, 15 species have been included within *Solicorynespora,* with species circumscribed primarily based on conidial shape, size, septation, ornamentation, lateral appendage and presence or absence of a rostrum (Castañeda 1996, Castañeda et al. 2004, McKenzie 2010, Ma et al. 2011b).

The forests of southern China have a rich mycota, and many lignicolous fungi have been discovered there (Dai & Cui 2006, Yuan & Dai 2009, Zhang et al. 2009, Cui et al. 2011, Ma et al. 2011a). During ongoing surveys of conidial

fungi associated with woody debris in tropical or subtropical forests of southern China, several hyphomycete species were collected on dead branches. The present paper deals with four species clearly related to the genus *Solicorynespora*. Three are new to science while the fourth is a new record for China.

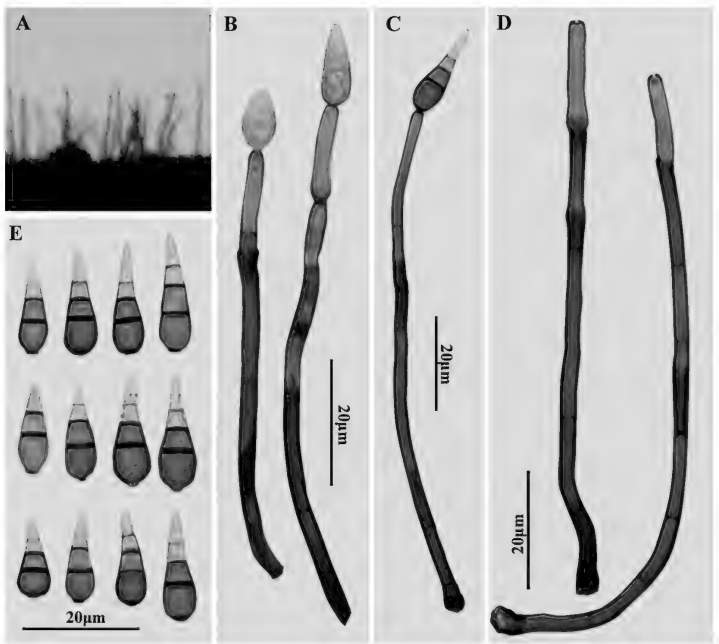

FIG. 1. *Solicorynespora ligustri*. A. Colonies on natural substratum. B. Conidiophores with developing conidia. C. Conidiophore with terminal conidium. D. Conidiophore apices showing conidiogenous cells and percurrent extensions. E. Conidia.

Solicorynespora ligustri Jian Ma & X.G. Zhang, **sp. nov.** FIG. 1

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Differs from *Solicorynespora zapatensis* and *S. calophylla* in smooth conidia that lack a constriction at the septa.

TYPE: China, Hainan Province: Natural Reserve of Liulianling, on dead branches of *Ligustrum quihoui* Carrière (*Oleaceae*), 5 Dec 2009, J. Ma, (**Holotype** HSAUP H5247; **isotype** HMAS 146078).

ETYMOLOGY: in reference to the host genus, *Ligustrum*.

Conidial fungi. Colonies on natural substrate effuse, brown to dark brown, hairy. Mycelium partly superficial, partly immersed in the substratum, composed of branched, septate, pale brown to brown, smooth-walled hyphae, 1–2.5 µm thick. Conidiophores distinct, single or in groups, erect, straight or flexuous,

unbranched, smooth, septate, brown to dark brown, 52–97 µm long, 2.5–4.5 µm wide. Conidiogenous cells monotretic, integrated, terminal, cylindrical, brown, smooth, 11–17 µm long, 2–3.5 µm wide, with 0–2 cylindrical percurrent extensions. Conidia solitary, dry, acrogenous, obclavate or obpyriform, mostly 3-euseptate, rarely 2-euseptate, smooth, the lower two cells brown, other cells pale brown to subhyaline, 13–22 µm long, 4.5–6 µm wide in the widest part, tapering to 1.5–2.5 µm near the apex, 1.5–2.5 µm wide at the truncate base.

Comments – Among the known *Solicorynespora* species, *S. ligustri* bears some resemblances to *S. zapatensis* and *S. calophylli* (Hol.-Jech. & R.F. Castañeda) R.F. Castañeda & W.B. Kendr. (Castañeda & Kendrick 1990) in conidial shape, but the conidia of *S. ligustri* are smaller than in *S. zapatensis* (20–27 × 15–17 µm) and longer and with more septa than in *S. calophylli* (11–16 µm long, 2-septate). *Solicorynespora zapatensis* and *S. calophylli* are additionally distinguished by having roughened conidia with constrictions at the septa.

Solicorynespora machili Jian Ma & X.G. Zhang, **sp. nov.** Fig. 2

MycoBank MB 563283

Differs from *Solicorynespora zapatensis* in producing smaller conidia with pigmented lower cells, from *S. calophylli* in having larger conidia, and from *S. ligustri* by its wider conidia with verrucose wall.

Type: China, Guangdong Province: Mount Dinghu, collected on dead branches of *Machilus chinensis* (Benth.) Hemsl. (*Lauraceae*). 18 Oct 2010, J. Ma, (**Holotype** HSAUP H5427; **isotype** HMAS 146079).

Etymology: in reference to the host genus, *Machilus*.

Conidial fungi. Colonies on natural substrate effuse, brown to dark brown, hairy. Mycelium partly superficial, partly immersed in the substratum, composed of branched, septate, pale brown to brown, smooth-walled hyphae, 1–2.5 µm thick. Conidiophores distinct, singly or in groups, erect, straight or flexuous, unbranched, smooth, septate, brown to dark brown, 76–110 µm long, 3.5–4.5 µm wide. Conidiogenous cells monotretic, integrated, terminal, brown, smooth, 10–16 µm long, 3.5–4.5 µm wide, with 0–1 lageniform percurrent extension. Conidia solitary, dry, acrogenous, obclavate or obpyriform, 3-euseptate, the lower two cells verrucose, brown to dark brown, other cells smooth, pale brown to subhyaline, 16–24 µm long, 7–8.5 µm wide in the widest part, tapering to 1.5–2.5 µm near the apex, 2–2.5 µm wide at the truncate base.

Comments – *Solicorynespora machili* resembles *S. zapatensis*, *S. calophylli* and *S. ligustri* in conidial shape. However, *S. zapatensis* can be distinguished by its distinctly larger conidia (20–27 × 15–17 µm) with unpigmented lower two cells, while *S. calophylli* has smaller (11–16 × 5–7 µm) 2-euseptate conidia. *Solicorynespora ligustri* is differentiated by its wider (4.5–6 µm) conidia with verrucose walls.

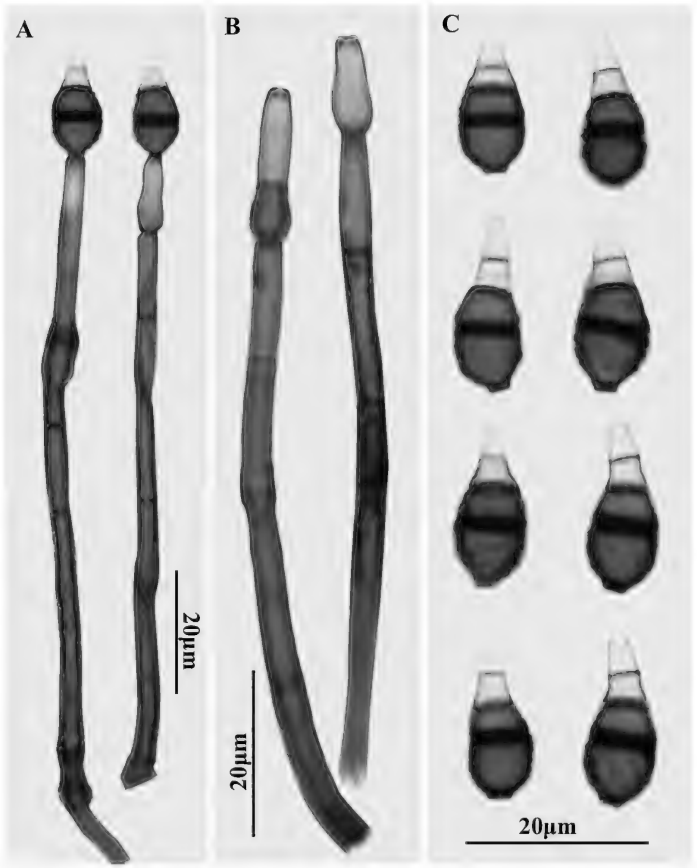

Fig. 2. *Solicorynespora machili*. A. Conidiophores with terminal conidia. B. Conidiophore apices showing conidiogenous cells and percurrent extension. C. Conidia.

Solicorynespora cryptocaryae Jian Ma & X.G. Zhang, **sp. nov.** Fig. 3

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Differs from *Solicorynespora mulanjeensis* and *S. pseudolmediae* in slightly wider conidia with more septa, and further differs from *S. mulanjeensis* and *S. pseudolmediae* in having shorter or longer conidia, respectively.

Type: China, Guangdong Province: Liuxihe National Forest Park, on dead branches of *Cryptocarya chinensis* Hemsl. (*Lauraceae*). 20 Oct 2010, J. Ma, (**Holotype** HSAUP H5367-2; **isotype** HMAS 146087).

Etymology: in reference to the host genus, *Cryptocarya*.

Conidial fungi. Colonies on natural substrate effuse, brown, hairy. Mycelium mostly immersed in the substratum, composed of branched, septate, pale brown

to brown, smooth-walled hyphae, 1.5–3 µm thick. Conidiophores distinct, single or in groups, erect, straight or flexuous, unbranched, smooth, septate, brown to dark brown, 86–140 µm long, 4.5–6.5 µm wide. Conidiogenous cells monotretic, integrated, terminal, brown, smooth, 13–21 µm long, 4–5.5 µm wide, with 0–1 cylindrical percurrent extension. Conidia solitary, dry, acrogenous, obclavate or ellipsoidal, gradually tapered to an obtuse paler apex, 6–12-euseptate, smooth, brown, 32–55 µm long, 10–13 µm wide in the widest part, 3.5–4 µm wide at the truncate base.

Comments – *Solicorynespora cryptocaryae* superficially resembles *S. mulanjeensis* (B. Sutton) R.F. Castañeda et al. (Castañeda et al. 2004) and *S. pseudolmediae* (Castañeda & Kendrick 1990). However, the conidia of *S. mulanjeensis* are longer (56–71 µm), and those of *S. pseudolmediae* are shorter (16–29 µm). Moreover, *S. mulanjeensis* (conidia 10–12.5 µm wide, 5–8-euseptate) and *S. pseudolmediae* (conidia 8.5–12 µm wide, 2–5-euseptate) are further distinguished by their slightly narrower conidia with fewer septa.

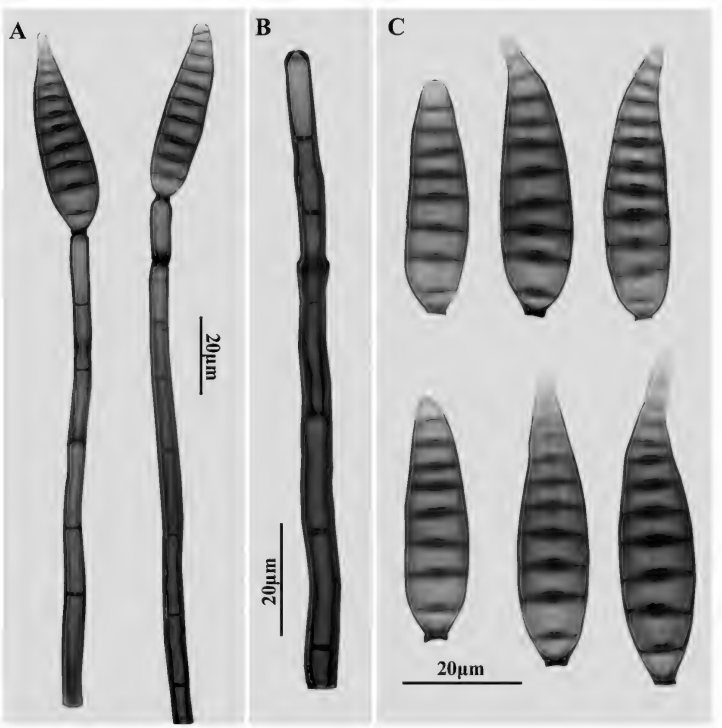

Fig. 3. *Solicorynespora cryptocaryae*. A. Conidiophores with terminal conidia, and conidiophore apices showing conidiogenous cells and percurrent extension. B. Conidiophore. C. Conidia.

Solicorynespora pseudolmediae (R.F. Castañeda) R.F. Castañeda & W.B. Kendr., Univ. Waterloo Biol. Ser. 33: 43, 1990. FIG. 4

≡ *Sporidesmium pseudolmediae* R.F. Castañeda, Rev. Jard. Bot. Nac. 5: 66. 1984.

≡ *Corynespora pseudolmediae* (R.F. Castañeda) Hol.-Jech., Česká Mykol. 40: 145. 1986.

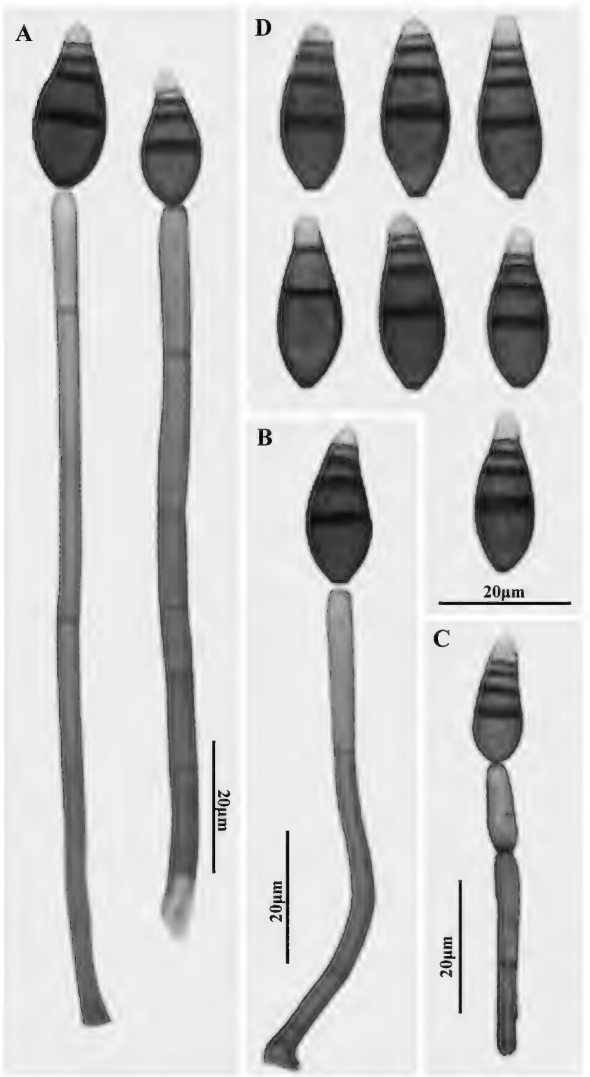

FIG. 4. *Solicorynespora pseudolmediae*. A. Conidiophores with terminal conidia. B. Conidiophore and conidium. C. Conidiophore with terminal conidium, and conidiophore apex showing conidiogenous cells and percurrent extension. D. Conidia.

Conidial fungi, hyphomycetes. Colonies on natural substrate effuse, dark brown, hairy. Mycelium partly superficial, partly immersed in the substratum, composed of branched, septate, pale brown to brown, smooth-walled hyphae, 1.5–3.5 µm thick. Conidiophores distinct, single or in groups, erect, straight or slightly flexuous, unbranched, smooth, brown, 40–170 µm long, 4–5.5 µm wide at the base, 4–5 µm wide in the middle, 3.5–4.5 µm wide at the apex, 2–5-septate, sometimes with 1–2 enteroblastic percurrent extensions. Conidiogenous cells monotretic, integrated, terminal, cylindrical, brown, smooth, 14–26 µm long, 3.5–5 µm wide. Conidial secession schizolytic. Conidia solitary, dry, acrogenous, obclavate or obpyriform, 2–5-euseptate (usually 4), sometimes slightly constricted at the speta, smooth, brown, with 1–2 upper cells pale brown or subhyaline, 22–32 µm long, 9–13 µm wide in the widest part, tapering to 3–4.5 µm wide near the apex, 2–3 µm wide at the truncate base.

Specimen examined: **CHINA, Yunnan Province**: Tropical Botanical Garden of Xishuangbanna, on dead branches of unidentified plant, 12 Oct 2007, J. Ma, (HSAUP H5286, HMAS 146088).

Comments –This species was originally assigned to *Sporidesmium* Link and subsequently transferred to *Corynespora* (Holubová-Jechová & Mercado 1986) due to its monotretic conidiogenous cells. Castañeda & Kendrick (1990) established the genus *Solicorynespora* and moved this species into the new genus based on its euseptate conidia. *Solicorynespora pseudolmediae* is morphologically similar to *S. zapatensis* in conidial shape, but differs from the latter in conidial size, wall ornamentation, and the number of septa. Comparing our collection with the type material in Castañeda's description (Castañeda 1984), the conidia of our specimen are slightly larger (22–32 × 9–13 µm vs. 16–29 × 8.5–12 µm) and the conidiophores are shorter (40–170 µm vs. 50–275 µm). Despite these minor differences, we believe they are basically the same species.

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Two new species of *Endophragmiella* from southern China

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Abstract —*Endophragmiella gardeniae* sp. nov., on dead branches of *Gardenia hainanensis,* and *E. machili* sp. nov., on dead branches of *Machilus grijsii,* are described and illustrated. They differ from other described *Endophragmiella* species in their obclavate or fusiform conidia with branches that developed a *Selenosporella*-like synanamorph. The specimens are deposited in Herbarium of Shandong Agricultural University, Plant Pathology (HSAUP) and Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences (HMAS).

Key words — anamorphic fungi, taxonomy

Introduction

Sutton (1973) established *Endophragmiella* for two species, *E. pallescens* B. Sutton (the type species) and *E. canadensis* (Ellis & Everh.) B. Sutton. Subsequently, the genus was emended by Hughes (1979), who gave a very detailed account of conidiogenesis and generic concepts. *Endophragmiella* is characterized by monoblastic, integrated, terminal, determinate or percurrently extending conidiogenous cells on distinct, branched or unbranched conidiophores, and solitary, acrogenous, septate conidia with rhexolytic conidial secession. To date, 79 species and one variety have been recognized within the genus, all from temperate to tropical areas (Wu & Zhang 2005, Chen et al. 2008, Brackel & Markovskaja 2009, Castañeda Ruiz et al. 2010, Leão-Ferreira & Gusmão 2010). The species are differentiated primarily by conidial shape, size, septation, pigmentation, ornamentation, and presence or absence of a rostrum (Sutton 1973, Hughes 1979, Wu & Zhuang 2005).

During ongoing surveys of saprobic microfungi from forests of southern China, two interesting anamorphic species were collected from dead branches. The characters of their conidia and conidiogenesis suggest

they are appropriately disposed in the genus *Endophragmiella*, although morphologically distinct from all previously known species. Therefore, they are described here as new to science.

Taxonomy

Endophragmiella gardeniae Jian Ma & X.G. Zhang, **sp. nov.** Fig. 1

MycoBank MB 519223

Differs from all other *Endophragmiella* species except *E. machili,* in producing conidia with branches that develop a *Selenosporella*-type anamorph.

Type: China, Hainan Province: tropical forest of Bawangling, on dead branches of *Gardenia hainanensis* Merr. (*Rubiaceae*), 11 Dec 2009, J. Ma (**holotype** HSAUP H5149; **isotype** HMAS 146102).

Etymology: in reference to the host genus, *Gardenia*.

Colonies on natural substrate effuse, brown, hairy. Mycelium partly superficial and partly immersed in the substratum, composed of branched, septate, pale

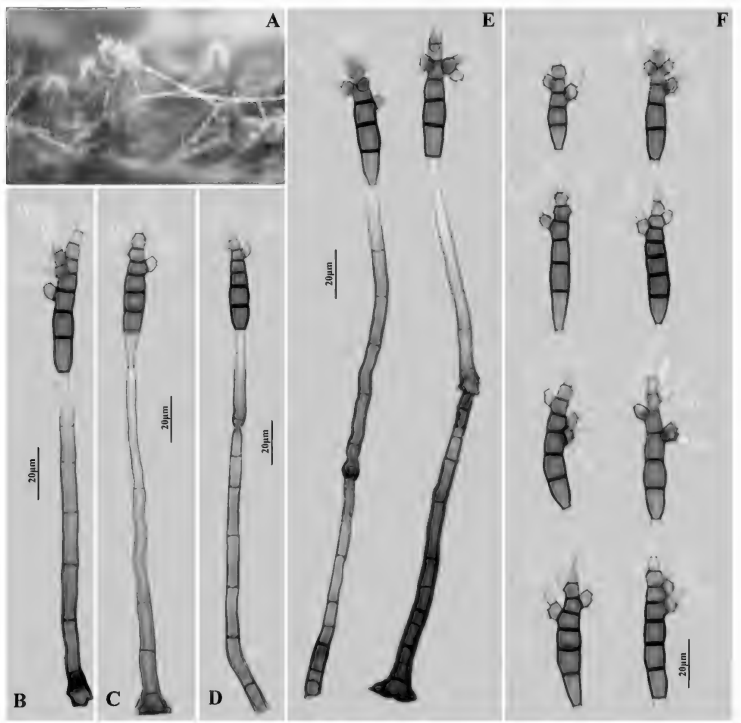

Fig. 1. *Endophragmiella gardeniae*. A. Colonies on natural substratum. B. Rhexolytic secession of conidium leading to a breakage of the conidiophore apex. C–E. Conidiophores with terminal conidia. In D–E conidiophores showing conidiogenous cells and percurrent extension. F. Conidia with branches that develop a *Selenosporella*-like synanamorph.

brown, smooth-walled hyphae, 1–3 µm thick. Conidiophores distinct, single or in groups, unbranched, cylindrical, erect, straight or flexuous, smooth, septate, brown to dark brown, 85–210 µm long, 4–7.5 µm wide, sometimes with 1 cylindrical percurrent extension. Conidiogenous cells monoblastic, integrated, terminal, indeterminate, cylindrical, smooth, pale brown to brown, 9–27 µm long, 4–5.5 µm wide. Conidial secession rhexolytic. Conidia acrogenous, solitary, obclavate or fusiform, 5–7-euseptate, slightly constricted at the septa, smooth, brown, apical cell pale brown to subhyaline, 41–66 µm long, 8–10.5 µm wide in the widest part, 2.5–4.5 µm wide at the truncate base, with a small collar of conidiogenous cell well, apical portion with branches that developed a *Selenosporella*-like synanamorph.

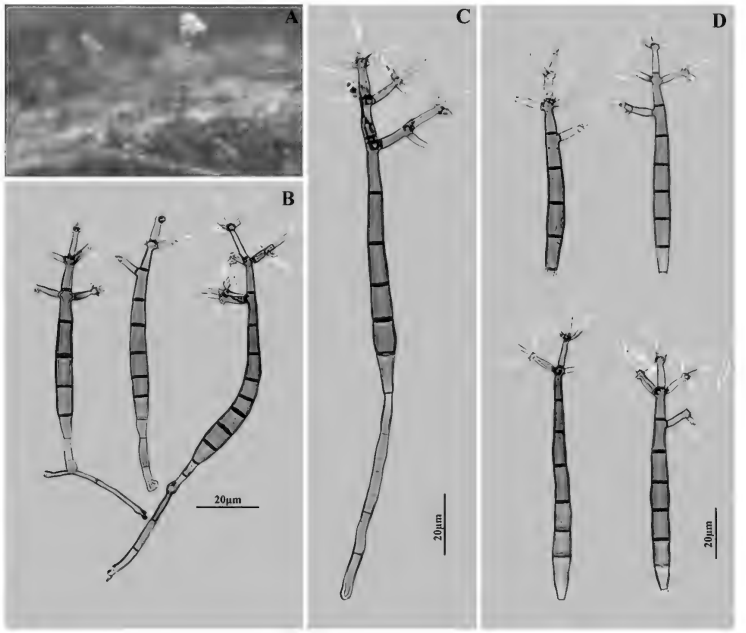

Fig. 2. *Endophragmiella machili*. A. Colonies on natural substratum. B–C. Conidiophores and conidia. D. Conidia with branches that develop a *Selenosporella*-like synanamorph.

Endophragmiella machili Jian Ma & X.G. Zhang, **sp. nov.** Fig. 2

MycoBank MB 519224

Differs from *Endophragmiella gardeniae* in its longer conidia with more septa.

Type: China, Fujian Province: subtropical forest of Mount Wuyi, on dead branches of *Machilus grijsii* Hance (*Lauraceae*), 15 Aug 2009, J. Ma (**holotype** HSAUP H5108–3; **isotype** HMAS 146103).

Etymology: in reference to the host genus, *Machilus*.

Colonies on natural substrate effuse, brown, hairy. Mycelium partly superficial and partly immersed in the substratum, composed of branched, septate, pale brown, smooth-walled hyphae, 1.5–3 µm thick. Conidiophores distinct, single or in groups, unbranched, determinate, cylindrical, erect, straight or flexuous, smooth, septate, brown, 4–57 µm long, 3–4.5 µm wide. Conidiogenous cells monoblastic, integrated, terminal, cylindrical, smooth, pale brown to brown, 7–14 µm long, 3–4 µm wide. Conidial secession rhexolytic. Conidia acrogenous, solitary, obclavate, straight or curved, 6–10-euseptate, sometimes slightly constricted at the septa, smooth, brown, apical cell pale brown to subhyaline, 93–135 µm long, 6–9 µm wide in the widest part, 2.5–4 µm wide at the truncate base, with a small collar of conidiogenous cell wall, apical portion with branches that developed a *Selenosporella*-like synanamorph.

Discussion

Endophragmiella gardeniae and *E. machili* are unlike the taxa hitherto placed in *Endophragmiella* in that their conidia have branches that develop a *Selenosporella*-like synanamorph. The initial thought was to introduce a new genus to accommodate these two species. However, Drs. Heredia and Arias showed us pictures of an *Endophragmiella* sp. (on decaying wood, Mexico, Veracruz, Acajete Municipal, Mesa de la Yerba, 19°33′N 97°01′W, 5 Dec. 2010, coll. G. Heredia & R.M. Arias) with many states of conidial development that are clearly the same as these two species. Therefore, we place them as new species in the genus *Endophragmiella*.

Endophragmiella gardeniae and *E. machili* bear some resemblance to *E. socia* (M.B. Ellis) S. Hughes (Hughes 1979) and *E. fagicola* P.M. Kirk (Kirk 1981) in conidial shape. However, the conidia of *E. machili* are longer and narrower than those of either *E. socia* (36–50 × 10.8–15.3 µm, 6–11-septate, usually 7) or *E. fagicola* (70–90 × 11–17 µm, usually 5-septate). *Endophragmiella gardeniae* also differs in conidial size and septation from *E. socia* and *E. fagicola*. In addition, *E. gardeniae* and *E. machili* produce conidia with branches that developed a *Selenosporella*-like synanamorph, a feature not found in *E. socia* and *E. fagicola*. *Endophragmiella gardeniae* differs from *E. machili* in its shorter conidia with fewer septa.

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Alveariospora, a new anamorphic genus from trichomes of *Dimorphandra mollis* in Brazil

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Abstract — A dematiaceous fungus associated with trichomes on leaflets of *Dimorphandra mollis* was collected during a survey of fungi associated with the genus *Dimorphandra* (*Fabaceae*). The host tree is endemic to the Brazilian Cerrado. Although widely distributed there, this plant has been neglected in mycological studies, since no fungus has ever been recorded in association with it. The new fungus produces large alveariform (skep or beehive-shaped), muriform, dictyosporous, distoseptate, verruculose conidia from large, thickened, dark washer-like conidiogenous loci. Among previously described anamorphic genera, this fungus is somewhat similar to *Annellophragmia, Annellosympodia*, *Briansuttonia*, *Dictyospiropes*, and *Veracruzomyces* as well as *Spiropes dictyosporus* but has some significant differences from those taxa. Hence, the new genus *Alveariospora* (type species: *A. distoseptata* sp. nov.) is proposed for this fungus.

Key words — fungal survey, taxonomy, tropical fungi

Introduction

Dimorphandra mollis Benth. (*Fabaceae*: faveira, faveiro-do-cerrado, falso barbatimão) is a plant native to the Cerrado. This species is adapted to conditions of low rainfall, developing foliage, flowering, and fruiting in the wet season and losing its leaves in the dry season (Lorenzi 2002). The fruits are utilized to extract rutin and other glycoside flavonoids for the pharmaceutical industry (Ferreira et al. 2001; Lorenzi 2002). It has potential for exploitation as a source of gum for the food industry (Panegassi et al. 2000). Although *D. mollis* is a common component of the cerrado flora — an ecosystem that in recent decades has been intensively explored by Brazilian mycologists (e.g., Rezende & Dianese 2003, Dornelo-Silva & Dianese 2004, Hernández-Gutiérrez & Dianese 2009, Pereira-Carvalho et al. 2010) — no attention has been paid to the mycobiota of this host plant and no fungi have ever been recorded on *D. mollis*.

In July 2009, an exploratory survey of the mycodiversity of *Dimorphandra* spp. was initiated, and the first brief survey to the municipalities of Paraopeba and Caetanópolis (Minas Gerais, Brazil) has already yielded several fungi of interest. One interesting fungus, found colonizing *D. mollis* trichomes, is described and discussed herein.

Material & methods

Samples of *D. mollis* foliage were collected, dried in a plant press, and taken to the lab. After examination, selected leaflets bearing fungus colonies were deposited in the local herbarium (Herbarium VIC). Isolations were attempted by direct spore transfer onto plates containing VBA medium (Pereira et al. 2003) with the help of a fine needle. Fungal structures were removed from fresh leaves and mounted in lactophenol. Observations, measurements, and illustrations were carried out with an OLYMPUS BX 51 light microscope fitted with a digital camera (EVOLT E330) and a drawing tube. Wherever possible, 30 measurements of each structure were taken.

Taxonomy

Alveariospora Meir. Silva, R.F. Castañeda, O.L. Pereira & R.W. Barreto, **anam. gen. nov.**

MycoBank 518826

Cellulae conidiogenae primo monoblasticae, deinde polyblasticae, indeterminatae, cum, cicatricibus primo apicalibus, deinde lateralibus, discoidibus, protuberantibus, nigris, incrassatis. Conidia alveariformia, muriformia, distoseptata, cum appendicibus cellularibus, praedita.

Type species: *Alveariospora distoseptata* Meir. Silva et al.

Etymology: Latin, *alvearium*, meaning beehive, skep; and *spora,* meaning spores.

Anamorphic fungi. Colonies on the natural substrate effuse, hairy, brown, olivaceous or black. Mycelium partly superficial and immersed. Conidiophores distinct, single, unbranched, septate, brown or olivaceous, smooth. Conidiogenous cells integrated, terminal, at first with a single terminal conidiogenous locus, then indeterminate, polyblastic, with successive sympodial but rectilinear proliferation, rupturing the outer wall around each scar, resulting in a lateral displacement of scars, leaving conspicuous circumferential annular fringes of the torn wall. Conidiogenous loci evident, lenticular, protuberant, thickened and black, conidial secession schizolytic. Conidia solitary, ellipsoidal, oval to broadly navicular, dictyoseptate, distoseptate, verruculose or smooth, brown or dark brown, conspicuously cicatrized at the base, with a cellular, cylindrical or subulate, brown apical appendage.

Alveariospora distoseptata Meir. Silva, R.F. Castañeda, O.L. Pereira & R.W. Barreto, anam. **sp. nov.** Plates 1–4

MycoBank 518827

Differs from *Spiropes dictyosporus* in hyphal conidiomata and distoseptate conidia.

Type: Brazil, Minas Gerais, Paraopeba, Floresta Nacional de Paraopeba, on trichomes of *Dimorphandra mollis*, 10 Jul. 2009, M. Silva & O.L. Pereira (**Holotype**, VIC 31399).

Etymology: Latin, *distoseptata*, meaning distoseptate, having the individual cells each surrounded by a sac-like wall distinct from the outer wall.

Anamorphic fungus. Colonies on the natural substrate effuse, hairy, brown. Mycelium partly superficial and partly immersed, composed of septate, branched, smooth, brown hyphae. Conidiophores distinct, single, unbranched, erect, straight, 3–6-septate, dark brown, pale brown near the apex, smooth, 120–160 × 7–10 µm. Conidiogenous cells integrated, terminal, 22–45 × 6–10 µm, at first with a single terminal conidiogenous locus, then indeterminate, polyblastic, with successive sympodial but rectilinear proliferation, rupturing the outer wall around each scar, resulting in a lateral displacement of scars, leaving conspicuous circumferential annular fringes of the torn wall. Conidiogenous loci evident, lenticular, black, 6–8 µm diam., 2–4 µm thick, 3–7 per conidiophore. Conidial secession schizolytic. Conidia solitary, ellipsoidal to broadly navicular, dictyoseptate, distoseptate, verruculose, brown, 62–85 × 26–45 µm, with a conspicuous protuberant, melanized, lenticular hilum at the base, 7–8 µm diam., 3–4 µm thick, and with a cellular, cylindrical or slightly subulate, smooth terminal appendages, 27–42 × 2.5–4.5 µm, brown and pale brown to subhyaline at the end, sometimes surrounded by a hyaline, mucilaginous sheath. Teleomorph unknown. In culture, no conidial germination was observed, even after a period of four weeks.

Comments — The pattern of ontogeny in *Alveariospora* can be classified as conidial development type 17 (Kirk et al. 2008) (holoblastic, delimitation by 1 septum, schizolytic secession, maturation by diffuse wall-building, percurrent enteroblastic conidiogenous cell extension, followed by conidial ontogeny by replacement apical wall-building; strongly melanized, each successive conidium seceding before the next percurrent elongation of the conidiogenous cell), but sometimes also holoblastic, sympodial proliferation occurring and two or more conidia are produced, a pattern of ontogeny classified as conidial development type 10 (holoblastic, regularly alternating with sympodial proliferation, maturation by diffuse wall-building and secession schizolytic).

The mode of rectilinear proliferation and the combination of conspicuous annular structures and thickened, darkened conidiogenous loci in *Alveariospora distoseptata* is very unusual and comparable only with *Annellophragmia* Subram. (Ellis 1971), *Annellosympodia* McTaggart et al. (McTaggart et al. 2007), and *Spiropes dictyosporus* Seifert & S. Hughes (Seifert & Hughes 2000). However, the last species, inhabiting a sooty mould in New Zealand, is characterized by synnematous conidiomata and euseptate conidia. *Annellophragmia* is differentiated by synnematous conidiomata and phragmosporous (but also distoseptate) conidia. *Annellosympodia* is characterized by its sporodochial

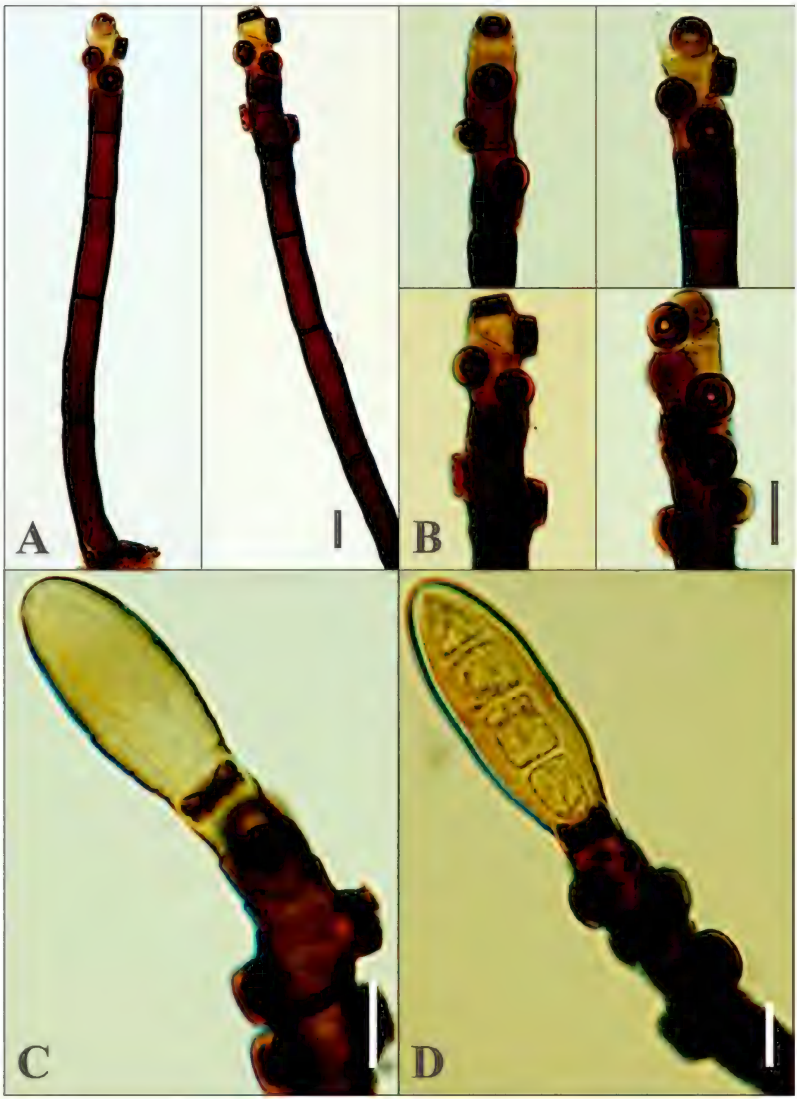

Plate 1. *Alveariospora distoseptata*. Macronematous conidiophores with polyblastic conidiogenous cells (A); detail of protuberant lenticular loci on enteroblastic percurrent proliferations (B); initial conidial formation (C) and initial conidial septation (D). Bars: 10 µm.

conidiomata with ampulliform, doliiform to obovoid conidiogenous cells, 0–1-euseptate conidia, and rhexolytic conidial secession. Among other known anamorphic genera, *Alveariospora* appears superficially similar to *Briansuttonia*

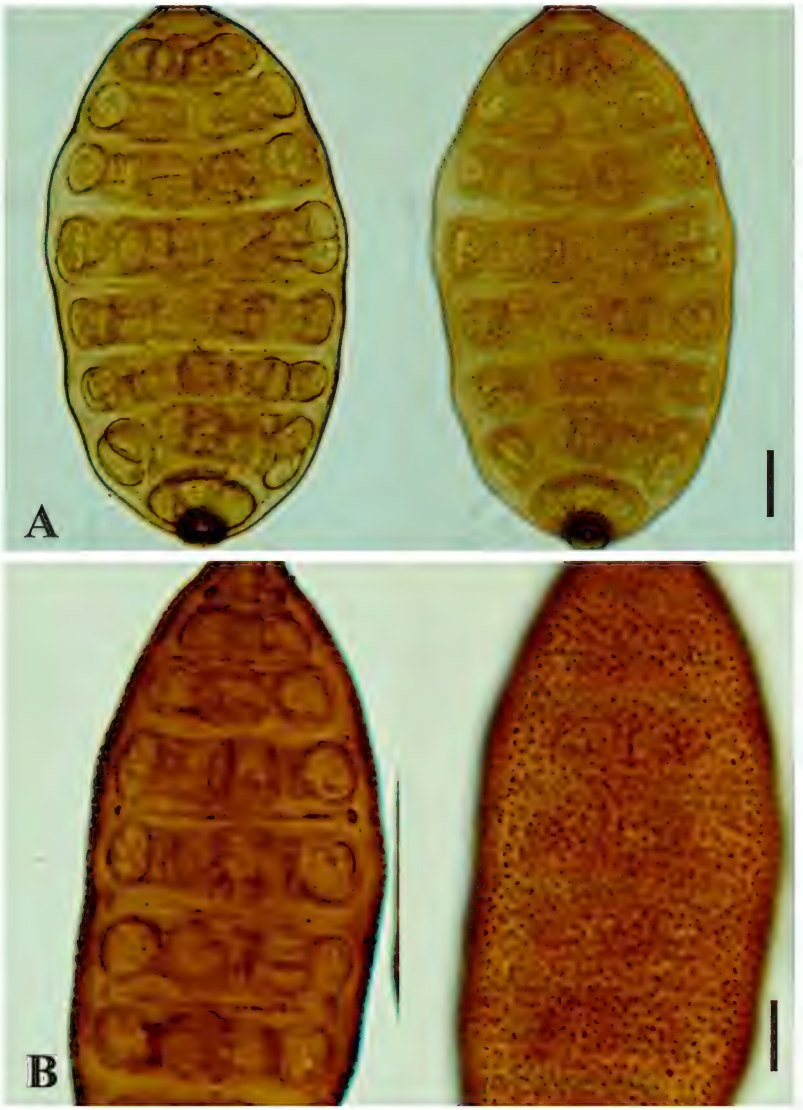

Plate 2. *Alveariospora distoseptata*. Detail view of muriform, distoseptate conidia (left) with verruculose wall (right) on young yellowish conidia (A) and reddish-brown mature conidia (B). Bars: 10 µm.

R.F. Castañeda et al. (Castañeda et al. 2004), *Dictyospiropes* M.B. Ellis (Ellis 1976), and *Veracruzomyces* Mercado et al. (Mercado-Sierra et al. 2002). *Briansuttonia* is characterized by monotretic, terminal, determinate or indeterminate with

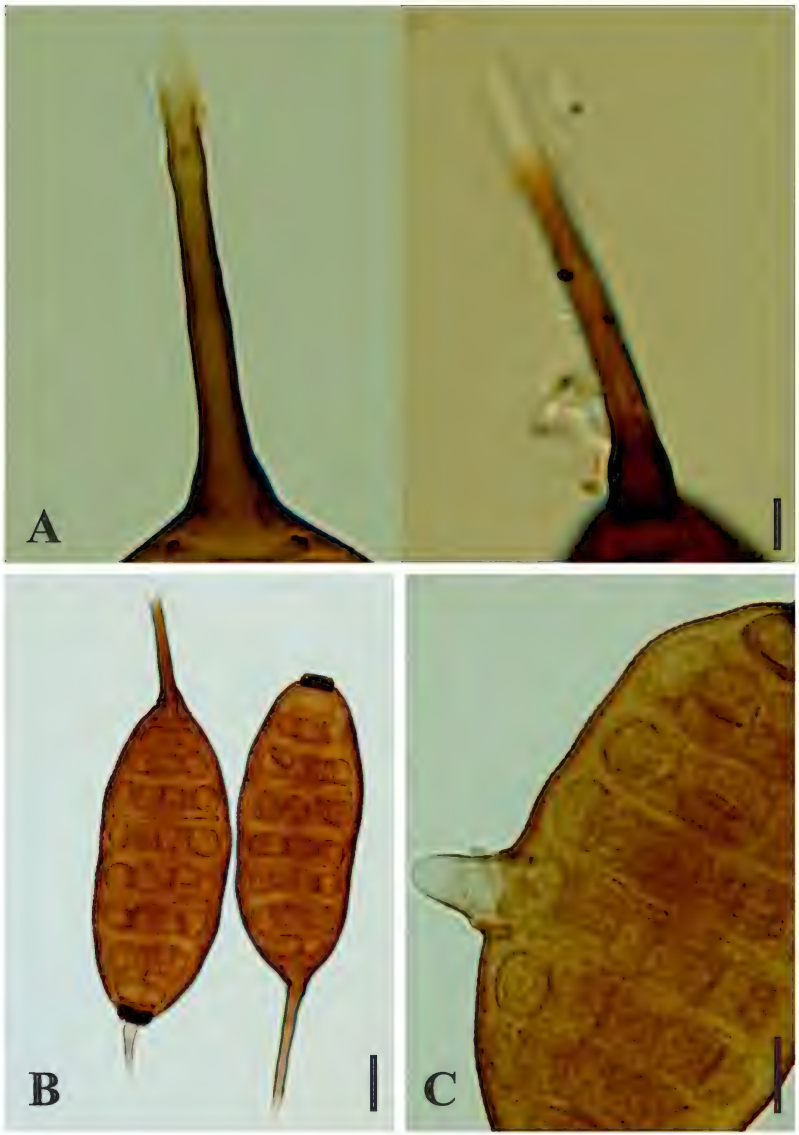

Plate 3. *Alveariospora distoseptata.* Conidial appendage ends, sometimes surrounded by a hyaline, mucilaginous tunica (A); germination of the conidia, through hilum (B) and laterally through wall (C). Bars: A = 5 µm; B = 20 µm; C = 10 µm.

enteroblastic percurrent conidiogenous cells. *Dictyospiropes* is quite distinct from *Alveariospora* by having polyblastic, sympodial conidiogenous cells with strongly melanized, lenticular conidiogenous loci lacking percurrent extensions,

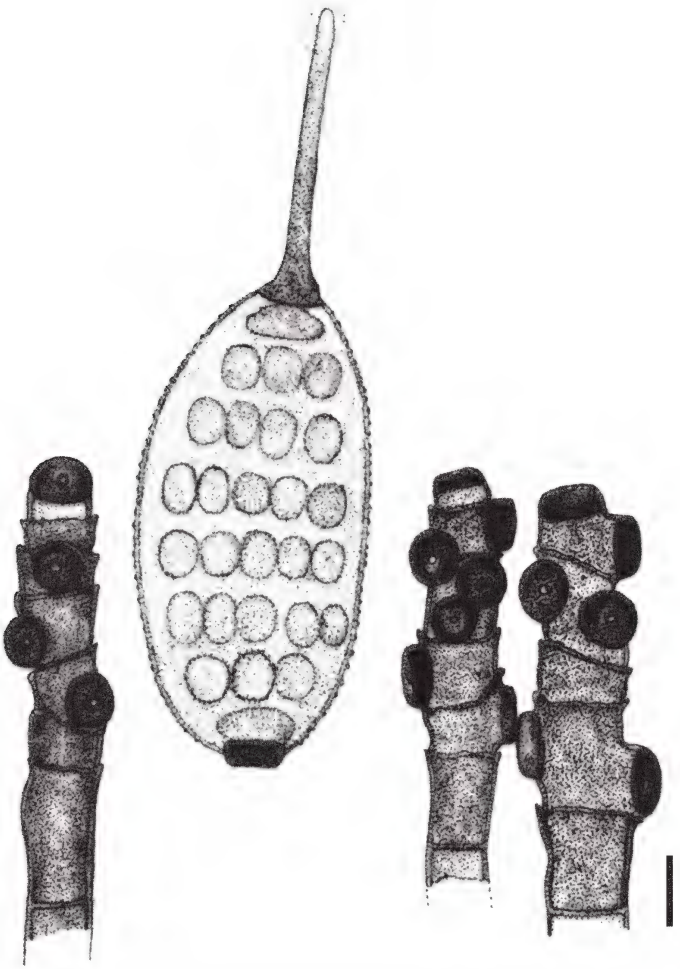

Plate 4. Drawing of *Alveariospora distoseptata* conidia and conidiogenous cells. Bar: 10 μm.

and euseptate-dictyoseptate conidia without strongly thickened and melanized basal hila. *Veracruzomyces* is clearly distinguished from *Alveariospora* by its monoblastic, percurrently proliferating conidiogenous cells without cicatrized conidiogenous loci.

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Intraornatosporaceae (*Gigasporales*), a new family with two new genera and two new species

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Abstract — A new family (*Intraornatosporaceae*), two new genera (*Intraornatospora*, *Paradentiscutata*), two new species (*P. bahiana*, *P. maritima*), and a new combination (*I. intraornata*) are presented in the *Gigasporales*. The genera, both with diagnostic introverted ornamentations on the spore wall, are distinguished by spore wall structure and germ shield characteristics. The new species, detected in NE Brazil, can be differentiated by their projections on the outer spore surface. Partial sequences of the LSU rRNA gene place both species next to *I. intraornata* in a monophyletic major clade related to *Gigasporaceae* and *Dentiscutataceae*.

Key words — *Glomeromycetes*, *Scutellospora*, molecular phylogeny, rDNA

Introduction

The phylum *Glomeromycota* has been extensively revised in recent years at all classification levels from class to species (e.g. Morton & Redecker 2001, Sieverding & Oehl 2006, Palenzuela et al. 2008, Goto et al. 2010, 2011, Oehl et al. 2011a,b,c). Major revisions were necessary within the order *Gigasporales* when separating *Gigasporaceae* species into four families (*Gigasporaceae*, *Scutellosporaceae*, *Racocetraceae* and *Dentiscutataceae*) and *Scutellospora* into seven genera when concomitant morphological and molecular spore analyses were undertaken (Oehl et al. 2008, 2010, 2011b,c). It was initially clear that *Dentiscutataceae,* in particular, was a heterogeneous family that needed more

detailed study (Oehl et al. 2008). Unfortunately, little progress has been made due to only a limited number of available isolates.

During recent studies on the diversity of the arbuscular mycorrhizal (AM) fungi (*Glomeromycota*) in Northeastern Brazil, *Dentiscutata colliculosa* B.T. Goto & Oehl, *Racocetra intraornata*, and two undescribed species were detected (Goto et al. 2009, 2010). All four species have introverted projections on the spore wall. The phylogenetic position of *R. intraornata,* however, remained unresolved while the two new species appeared to belong to the *Dentiscutataceae,* based on their spore formation on sporogenous cells and the presence of yellow brown to brown shields on the inner spore walls. Moreover, two major morphological characters separated these two new species within the *Dentiscutataceae* — the multiple-lobed structure of their openly organized shields and the presence of a tuberculate ornamentation on the inner surface of the hyaline middle spore wall. A multiple-lobed structure of openly organized shields has been detected only in the *Racocetraceae*, where shields are hyaline to sub-hyaline. Ornamentation on the inner surface of the middle wall is hitherto a unique character shared with no other species within the *Glomeromycota*. *Racocetra intraornata*, however, was understood to have a rather rudimentary germination shield where germ tube initiations are rarely found. The question therefore arose whether this species truly belongs to *Racocetra*, or might have a different phylogeny.

The main aim of this study was thus to analyze thoroughly and describe the two new species and to elucidate their phylogenetic position as well as that of *R. intraornata.*

Materials & methods

Study areas, soil sampling and soil parameters

One of the two new AM species was recovered in Serra da Jibóia, a fragment of the Atlantic Rainforest ('Mata Atlântica') in Santa Terezinha, Bahia State. The other species was found in several locations in the sand dunes of Mataraca, Paraíba State, (Souza et al. 2010, 2012) and in Natal, Rio Grande do Norte State. In the Serra da Jibóia, the soils were taken in September 2010, in Mataraca in January and August 2005, and in Natal in February 2010, as described in (Souza et al. 2010, 2012).

The Serra da Jibóia (12°51′S 39°28′W) is a forest fragment characterized by high plant diversity (Queiroz et al. 1996) located on a hillside surrounded by drier areas in transition to Caatinga (dry forest with trees and shrubs, deciduous during the dry season; Andrade-Lima 1964). Mean annual temperature is 17–28°C with 1200 mm of rain falling between April and July (Tomasoni & Santos 2003). The sand dunes in Mataraca are located at 6°28′–6°30′S 34°55′–34°57′W. The vegetation is typical of 'restinga,' a transition ecosystem between the primary coastal sand dunes and the tropical Atlantic Rainforest with physiognomy varying from tree-shrub to herbaceous plants (Oliveira-Filho & Carvalho 1993). The predominant geological formation is sandy-clay

sedimentary rocks, superimposed by fixed dunes. The climate is tropical-(semi-)humid (type Am of Köppen-Geiger, Kottek et al. 2006) with four months of dry season. Average annual temperature is 24–28°C and rainfall 1795 mm. The sand dunes in Natal represent one of the largest urban conservation areas with dune vegetation in Brazil. The 'Parque das Dunas' site (5°46′S and 35°12′W) also has typical 'restinga' vegetation. The climate is tropical rainy (type Am of Köppen) with a short dry period of four months. The mean annual temperature is 25.5 °C, and the mean annual precipitation is 1191 mm.

Soil pH (H_20) in Santa Terezinha was 4.6, organic C was 21.5 g kg^{-1} and available P (after Mehlich; Nelson et al. 1953) 2.0 mg kg^{-1}. In Mataraca, soil pH was 5.6, organic C 25.0 g kg^{-1} and available P 6.5 mg kg^{-1}. In Natal, pH was 5.6, organic C 16.6 mg kg^{-1}, and available P was 3.0 mg kg^{-1}. Soils in all three areas were sandy.

AM fungal bait cultures

The native AM fungal communities from Serra da Jibóia and Mataraca were cultured in bait (= trap) cultures over three consecutive cycles (three months each) using corn (*Zea mays* L.), peanut (*Arachis hypogaea* L.) and sunflower (*Helianthus annuus* L.) in 500 mL pots, filled with autoclaved sand-vermiculite substrate (1:1 w/w; 400 g per pot) mixed with the natural field soil as AM fungal inoculum (50 g per pot), in the greenhouse of the Department of Mycology, Universidade Federal de Pernambuco, Recife. Additionally, multiple glomerospores of the new species were separated and used as infective propagules in single species cultures on *Sorghum bicolor* (L.) Moench as described in Palenzuela et al. (2010), Tchabi et al. (2010), and Goto et al. (2011). The new Serra da Jibóia species was successfully propagated in a two-species culture together with *Ambispora appendicula*, and the sand dune species in a bait culture together with *Racocetra tropicana* (Goto et al. 2011). However, no single species cultures were obtained from the two fungi. Spores isolated from the cultures or directly obtained from the field samples were used for morphological and molecular analyses.

Morphological analyses

Spores of the two new species and *R. intraornata* were extracted according to Sieverding (1991) and thereafter mounted in polyvinyl-alcohol–lacto–glycerin (PVLG), in PVLG + Melzer's reagent, and in water (Brundrett et al. 1994, Spain 1990). Over 100 spores were examined per species. The germination shields of each species were carefully separated from their spores by applying pressure and movements to cover slides in PVLG. Species descriptions utilize spore and germination terminology for gigasporalean species defined by Oehl et al. (2008, 2010, 2011b), Silva et al. (2008), and Goto et al. (2010, 2011).

Molecular analyses

DNA was extracted from single spores, with three single spore extractions for each of the two new species and one for *R. intraornata*. Individual spores were placed on a slide in a drop (5–10 µl) of ultrapure water, crushed with a needle, and used directly in the PCR reactions.

The extracts served as templates for a semi-nested PCR using primers ITS3 (White et al. 1990) – 28G2 (Silva et al. 2006) and LR1 (van Tuinen et al. 1998) – 28G2 consecutively. The template for the second PCR reaction was a 1:50 dilution of the first product. PCR reactions were carried out in a volume of 50 µl, containing 75 mM Tris-HCl pH 8.8, 200

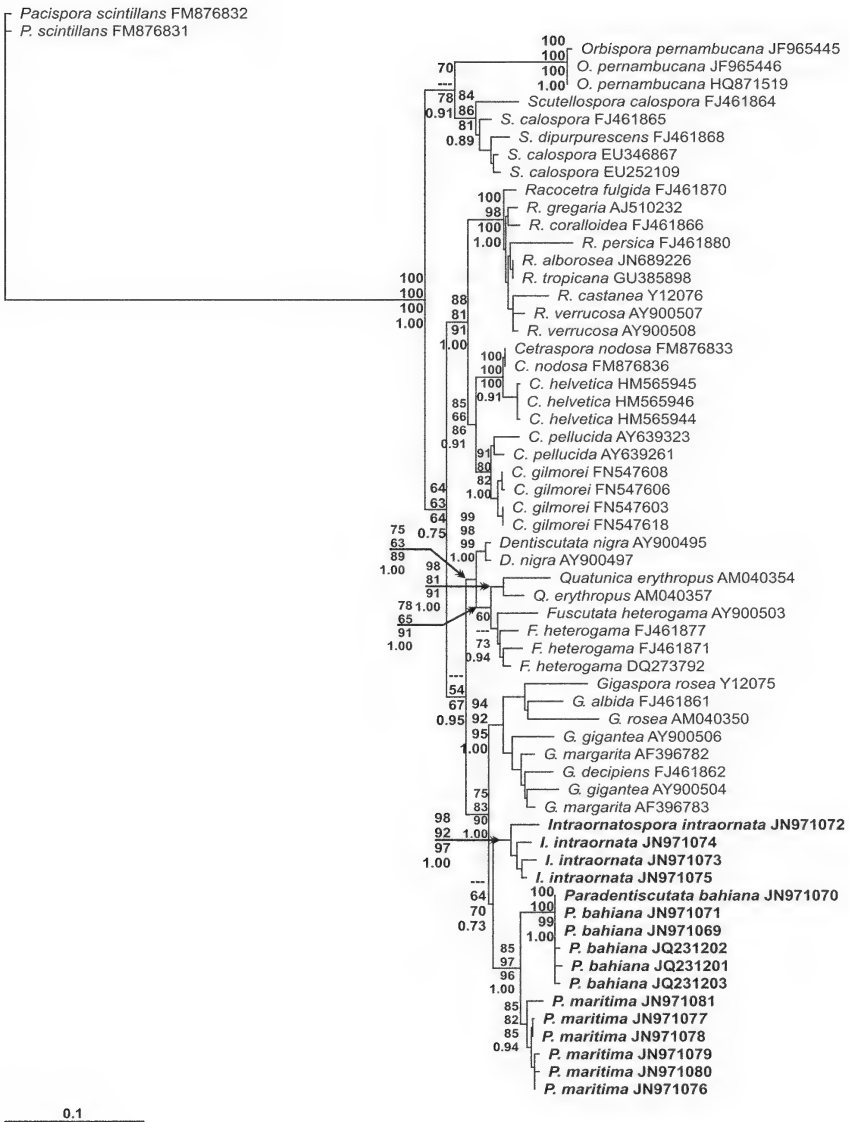


FIG. 1. Phylogenetic tree of the *Gigasporales* based on LSU rDNA analysis and rooted by *Pacispora scintillans*. Sequences are labeled with database accession numbers. Support values (from up to down) are from neighbor-joining (NJ), maximum parsimony (MP), maximum likelihood (ML) and Bayesian analyses, respectively. *Intraornatospora intraornata*, *Paradentiscutata bahiana* and *P. maritima* sequences are in bold. Only topologies with ≥50% bootstrap values are shown. (Consistency Index = 0.53; Retention Index = 0.79).

mM $(NH_4)_2SO_4$, 0.01% Tween 20, 2 mM $MgCl_2$, 200 µM each dNTPs, 1µM of each primer and 2 units of Taq™ DNA polymerase (Fermentas; Maryland, USA); cycling parameters were 5 min at 95°C (1 cycle), 45s at 94°C, 1 min at 55°C, 1 min at 72°C (40 cycles), and a final elongation of 7 min at 72°C followed the last cycle. The final amplicons (~690bp) were purified with a PureLink PCR Purification Kit (Invitrogen), sequenced directly or cloned with a CloneJET™ PCR Cloning kit (Fermentas; Carlsbad, USA) following the manufacturer's instructions and sequenced. Sequencing was provided by the Human Genome Research Center (São Paulo, Brazil).

Through a BLASTn query of the National Center for Biotechnology Information databases, we verified that the sequences obtained from *P. maritima*, *P. bahiana,* and *R. intraornata* affiliated with the *Gigasporales* (*Glomeromycota*) before phylogenetic analysis. The AM fungal sequences (partial LSU rRNA) obtained were aligned with other glomeromycotean sequences from GenBank using ClustalX (Larkin et al. 2007) and edited with BioEdit (Hall 1999). The sequences were deposited at GenBank under the accession numbers JN971069-JN971081 and JQ231201-JQ231203.

Maximum parsimony (MP) and neighbor joining (NJ) analyses with 1000 bootstrap replications were performed using the Phylogenetic Analysis Using Parsimony (PAUP) vers. 4 (Swofford 2003). Bayesian (two runs over 1×10^6 generations with a burnin value of 2500) and maximum likelihood (1000 bootstrap) analyses were executed, respectively, in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) and PhyML (Guindon & Gascuel 2003), launched from Topali 2.5. The model of nucleotide substitution (GTR + G) was estimated using Topali 2.5 (Milne et al. 2004). Sequences from *Pacispora scintillans* were used as an outgroup.

Results

Molecular analyses

The phylogenetic analyses generated by the LSU rRNA gene sequences (Fig. 1) revealed that the three species grouped in a monophyletic major clade most closely associated with the *Gigasporaceae* and *Dentiscutataceae* clade, with 75% bootstrap value for at least one phylogenetic method. The data further confirmed that the new genera and two new species conferred bootstrap values above 79%, thus supporting a new family and two new genera within the *Gigasporales* (Oehl et al. 2011b). We hereafter describe the two new species in one new genus and transfer *R. intraornata* from *Racocetra* to another, hitherto monospecific genus as the type species of the new family.

Taxonomic analyses

Intraornatosporaceae B.T. Goto & Oehl, **fam. nov.**

MycoBank MB 563599

Sporae ad cellulas sporogeneas, tunicis duabus vel tribus; ornamentatione introvertita in superficie interiore tunicae mediae (tribus tunicis) vel exterioris (duabus tunicis); scutellum germinale multilobatum, flavum-brunneum ad brunneum in sporis tribus tunicis, hyalinum vel subhyalinum duabus tunicis.

Type species: *Intraornatospora intraornata* (B.T. Goto & Oehl) B.T. Goto et al.

Sporocarps are unknown. Spores formed singly on bulbous sporogenous cells, terminally on a subtending hypha that arise from mycelial hyphae. Spores have 2 or 3 walls. The outer spore wall has 3–4 layers and inner wall 2–3 layers. Middle wall of 3-walled spores has an expanding outer and an inner layer with tuberculate ornamentation towards the inner wall. Outer wall of 2-walled spores also has introverted tuberculate ornamentation. Germination shield generally formed on the outer surface of the innermost wall or beneath a thin outer layer of the inner wall. Shields of triple-walled spores yellow-brown to brown, with 4–8(–10) wave-like lobed projections; folds separate the lobes on the shield, and each lobe may have a germ tube initiation from where the germ tubes arise and penetrate the outer wall. Shield in bi-walled spores, hyaline to light yellow, with 4–8 wave-like lobed projections; folds separate the lobes on the shield, which often appears immature, even in more aged spores.

Type genus: *Intraornatospora* B.T. Goto et al.
Other genus: *Paradentiscutata* B.T. Goto et al.

Intraornatospora B.T. Goto, Oehl & G.A. Silva, **gen. nov.** Figs 2–4

MycoBank MB 563600

Sporae ornamentatione introvertita in superficie interiore tunicae exterioris; scutellum germinale superficie tunicae interioris, multilobatum hyalinumque.

Type species: *Intraornatospora intraornata* (B.T. Goto & Oehl) B.T. Goto et al.

Etymology: Latin: *intra* (= inside), *ornata* (= ornamented), spora (= spore); referring to the position of the ornamentation on the spore in the type species.

Spores formed singly in soils, rarely in roots, on bulbous sporogenous cells that arise terminally on mycelial hyphae. The spores have two walls. The outer spore wall is generally triple-layered and continuous with the wall of the sporogenous cell. Inner surface of the outer spore wall has tuberculate or spiny projections. The inner wall is hyaline, 2–3 layered and forms de novo. A germination shield arises on the outer surface of the inner wall or beneath a thin outer layer of the inner wall; shield is hyaline to light yellow, generally oval to ellipsoid or subglobose, with several wave-like lobed projections forming the outer surface of the shield; folds separate the lobes on the shield. Germ tube initiations within the lobes often appear rudimentary and are rarely observed.

Intraornatospora intraornata (B.T. Goto & Oehl) B.T. Goto, Oehl & G.A. Silva, **comb. nov.** Figs 2–5

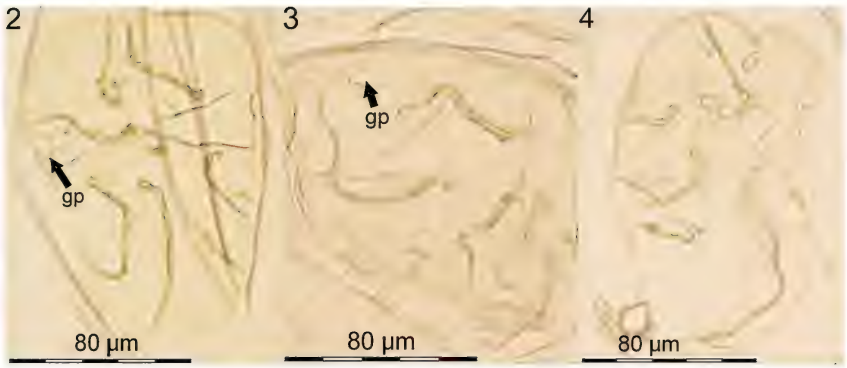
MycoBank MB 563601

≡ *Racocetra intraornata* B.T. Goto & Oehl, Mycotaxon 109: 485. 2009.

Paradentiscutata B.T. Goto, Oehl & G.A. Silva, **gen. nov.**

MycoBank MB 563602

Sporae ornamentatione introvertita in superficie interiore tunicae mediae; stratum exterius tunicae mediae expansivum; scutellum germinale, multilobatum, (flavo-)brunneum.



Figs 2–4. *Intraornatospora intraornata* (isotype, ZT Myc 775): typical, multiple-lobed germination shields; Germ pore (gp) as connection between shield and cell contents regularly visible when shield is in planar view (Figs 2–3); germ tube initiations rarely if ever visible.

Type species: *Paradentiscutata bahiana* Oehl et al.

Etymology: Latin: *para* (= equal), *dentata* (= dentate), *scutata* (= with shield); referring to the similarities with the germination shields of spores of *Dentiscutataceae*.

Sporocarps are unknown. Spores formed singly on bulbous sporogenous cells, terminally on subtending hypha that arise from mycelial hyphae. Spores have three walls; outer spore wall with 3–4 layers, middle wall with an expanding outer and a tuberculate ornamentation towards the inner wall, and an inner wall with 2–3 layers. Germination shields are yellow-brown to brown, as in *Dentiscutataceae*, but with 4–8(–10) wave-like lobed projections forming the outer surface of the shield; folds separate the lobes on the shield, and each lobe may have a germ tube initiation from where the germ tubes arise and penetrate the outer wall.

Paradentiscutata bahiana Oehl, Magna, B.T. Goto & G.A. Silva, **sp. nov.**

MycoBank MB 563603 Figs 5–20

Sporae aurantio-brunneae; stratum exterius verrucis, ≤ 1.0 µm in distantia.

Type: BRAZIL. Bahia State, Santa Terezinha, Serra de Jibóia, tropical rainforest fragment, [September 2010], [G.A. Silva], 92–9201 (**Holotype**, URM 83317); **Isotypes:** 92–9202 (URM 83318), 92–9203 (URM 83319), 9204–9212 (ZT Myc 7624).

Etymology: referring to Bahia State (NE Brazil), where the fungus was first found.

Glomerospores formed singly in soil, terminally on a subterminal bulbous suspensor cell (= 'sporogenous' cell). Glomerospores are dark orange brown to brown, globose, 190–260 µm in diameter, to subglobose, 190–265 × 190–240 µm, and have three walls (an outer, middle and inner wall). The spores become dark black brown to black when exposed to Melzer's reagent.

Outer wall (ow) is 3.6–6.0 µm thick in total and consists of 2(-3) layers: outermost wall layer (owl1) is hyaline to subhyaline to light yellow, semi-

persistent to persistent and 0.9–1.5 µm thick. It is covered with densely crowded irregular warts that are 0.8–2.0(–2.5) µm high and 1.0–2.1(–3.5) × 1.0–2.5(–5.0) µm wide. OWL2 is 2.1–3.4 µm thick, orange brown to brown, becoming dark orange brown to dark reddish brown when exposed to Melzer's reagent. OWL3 is concolorous and adherent with OWL2, very thin (0.6–1.1 µm), and generally not observed even in crushed spores.

MIDDLE WALL (MW) is hyaline to rarely light yellow, bi-layered and 4.8–6.8 µm thick when mounted in water. MWL1 is hyaline and 3.8–5.2 µm thick when mounted in water. In crushed spores, it expands to 9.0–16.0 µm and regularly expands further to 20–32(–56) µm in lactic acid based mountants. MWL1 may even become completely transparent making it difficult to detect due to even stronger swelling processes. MWL2 is hyaline becoming light yellow in older spores, 1.0–1.6 µm thick and bears fine tubes on its inner surface that are 0.5–1.1 µm high, 0.4–1.0 µm wide and 1.0–2.5 µm spaced. MWL1 stains purple black to black in Melzer's reagent.

INNER WALL (IW) is triple-layered, 2.8–4.5 µm thick bearing a germination shield on the outer surface. The outer IW layer (IWL1) is hyaline, semi-flexible, 0.6–1.1 µm thick and often wrinkles in crushed spores when separated from IWL2. The second layer (IWL2) is semi-flexible to unite, rarely 'amorph' when slightly expanding in PVLG based mounting, and is 1.6–2.2 µm thick. The innermost layer (IWL3) is relatively thin (0.6–1.2 µm thick), flexible, mostly tightly adherent to IWL2, and therefore generally difficult to observe. IWL2 and IWL3 stain yellow-pink to light purple in Melzer's reagent.

SPOROGENOUS CELL (SC) is globose to elongate, 32–50 µm long and 28–38 µm broad. It is concolorous with the spore wall. Two wall layers are visible on the young sporogenous cell, which are continuous with OWL1 and with laminated OWL2. OWL1 at sc is 0.4–1.2 µm thick, smooth and semi-persistent; OWL2 is 1.0–2.5 µm thick and persistent as long as sc remains attached on the spore. One to two 'hyphal pegs' may be rarely detected on the sporogenous cells. The sporogenous hypha attached to the cell is also bi-layered, 12–21 µm in diameter, tapering to 7–11 µm within 100–250 µm distance from the sporogenous cell. Within this distance, the sporogenous hyphal wall tapers from 1.5–2.2 µm to 1.0–1.7 µm, and 4–9 septa originating from OWL2 may be visible in variable distances within the sporogenous hypha. The ornamentation on OWL1 does not continue on the wall of the sporogenous cell.

GERMINATION SHIELD is yellow brown to brown, subglobose to oval to cardioid, to rarely ellipsoid or oblong, 140–200 × 121–180 µm in diameter, and multiple-lobed, generally with (4–)6–10 lobes. The shield is open-organized, as

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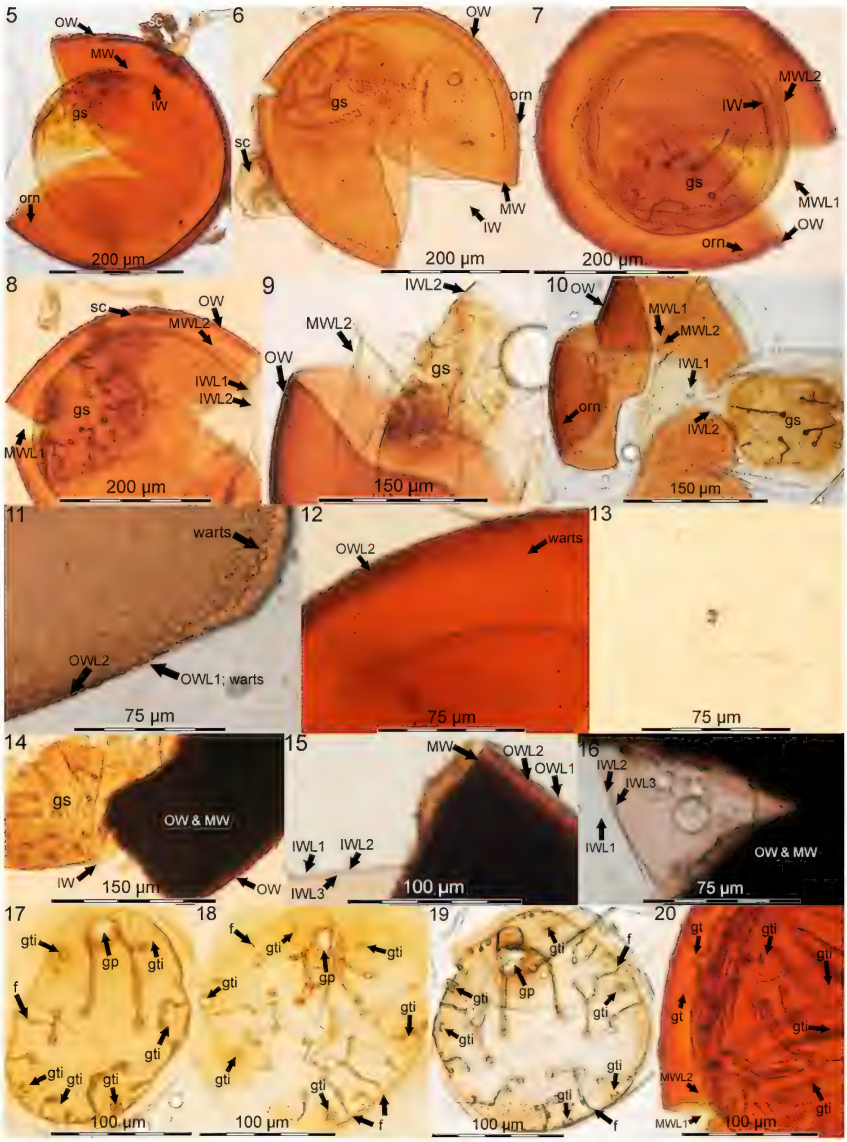
FIGS 5–20. *Paradentiscutata bahiana* (Type URM 83317–83319, ZT Myc 7624): **5–10**. Spores (crushed) formed on sporogenous cells (sc) with surface ornamentation (orn), showing outer wall (OW), middle wall (MW), inner wall (IW) and conspicuous, yellow brown to brown germination shields (gs). Each two layers of MW (MWL1-2) and IW (IWL1-2) visible. MWL1 expanding in lactic



acid based mountants; MWL2 with needle-tuberculate ornamentation. **11–12**. Verrucose surface ornamentation (warts) in cross and planar view, respectively. **13**. Ornamentation of MWL2 in planar view. **14–16**. Spore segments (crushed) in Melzer's reagent; expanding MWL1 stains dark purple to black purple. IW triple-layered (IWL1-3; IWL2 staining light purple to purple. **17–20**. Characteristic germination shields are open-organized with multiple wavy-lobes, mostly bearing, each, one germ tube initiation (gti) from where a germ tube (gt) emerges; lobes are separated by large folds (f), and a single germ pore (gp) connects the shield with the cell content.

known for species of *Racocetraceae*. Large folds (~11–45 µm long) arise from the shield wall separating the lobes. The one-layered shield wall and the folds are generally 1.2–1.8 µm thick. The shield periphery regularly appears slightly dentate until the germination has started. Each lobe may bear one rounded germ tube initiation (gti), 6.5–12.0 µm in diameter. The majority of the gti's may remain undetectable in young spores, becoming increasingly visible with age of spores. Single germination tubes may emerge from one, two to rarely three gti's during early germination, penetrating the OW and branching in the spore periphery within a short distance.

Arbuscular mycorrhiza forming (as proofed in dual-species cultures together with *Ambispora appendicula*, after staining in trypan blue).

Distribution: *Paradentiscutata bahiana* has been recovered only from natural tropical rainforest in Santa Terezinha, Serra da Jibóia, Bahia State.

Paradentiscutata maritima B.T. Goto, D.K. Silva, Oehl & G.A. Silva, **sp. nov.**

MycoBank MB 563604 Figs 21–31

Differt ad Paradentiscutata bahiana *in ornamentatione superficialis: stratum exterius papillis rotundatis, 3.0-8.0 µm in distantia.*

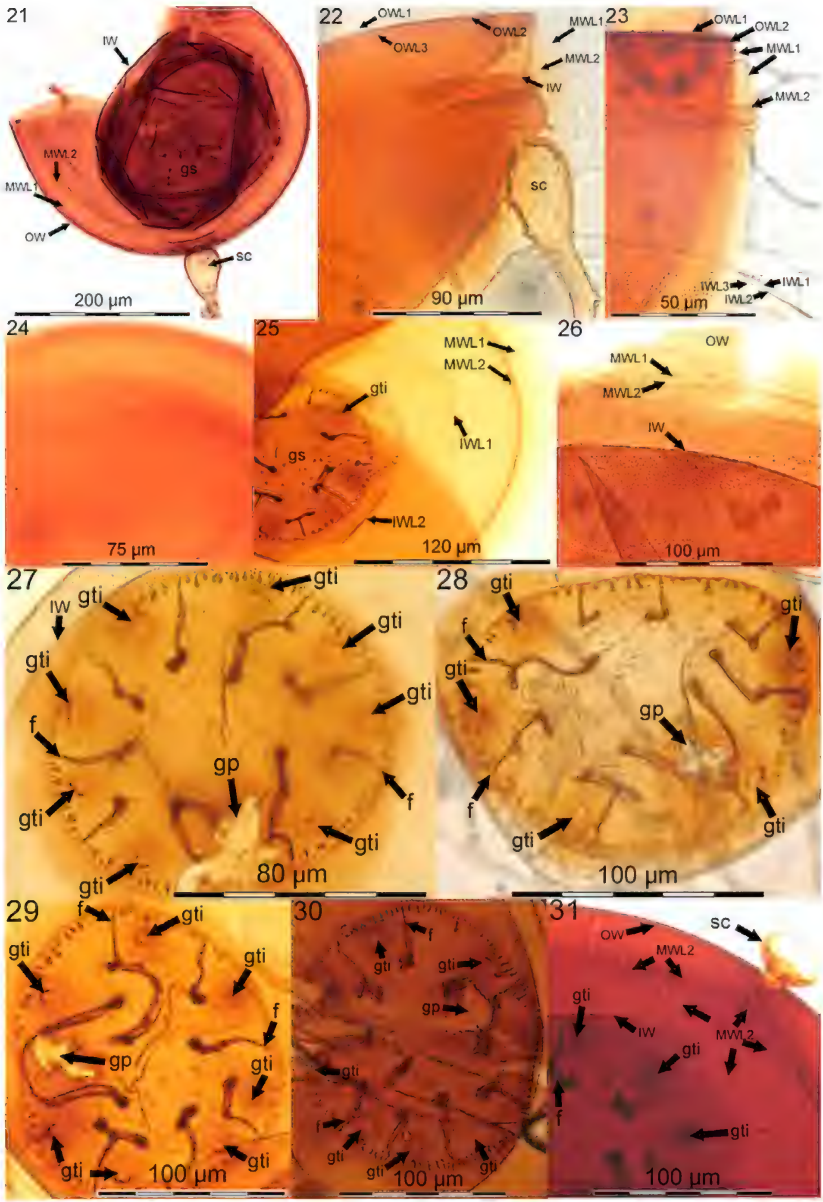
Type: BRAZIL. Paraiba State, Mataraca, Atlantic Rainforest biome, 'restinga' vegetation in sand dunes, [August 2005], [R.G. Souza], 93–9301 (**Holotype**, URM 83320); **Isotypes** 9302 (URM 83321), 9303 (URM 83322), 9304–9310 (ZT Myc 7625).

Etymology: Latin: *maritima* (=maritime, coastal), referring to occurrence in sand dune systems of the Atlantic coast.

Glomerospores formed singly in soils terminally on a subterminal or intercalary bulbous suspensor cell. Spores are globose (150–260 µm in diameter) to subglobose (145–250 × 165–280 µm), bright (yellowish) brown, orange brown to dark brown, with three walls: outer, middle, and inner (ow, mw, and iw).

Outer wall (ow) is three-layered: outermost wall layer (owl1) is 0.5–1.2 µm thick and has a papillae ornamentation with projections that are 1.5–2.5 µm high and 1.2–2.6 µm wide at base, and 3.0–8.0 µm apart. owl2 is yellow to orange, persistent, 2.5–3.8 µm thick and laminate. owl3 is 0.5–1.0 µm thick, tightly adherent to owl2 and especially difficult to distinguish in lactic acid-based mountants, also due to expansive mwl1 beneath. owl2 stains red to red-brown in Melzer's reagent. The straight pore channel at the spore base is about 2.5–4.2 µm broad and is generally closed by a plug formed by owl2.

Figs 21–31. *Paradentiscutata maritima* (Type URM 83320–URM83322, ZT Myc 7625): **21**. Spore (crushed) formed on sporogenous cell (sc) with three walls (ow, mw, iw) and germination shield (gs); iw staining purple in Melzer's reagent. Two layers of mw (mwl1-2) visible. **22–23**. Spore wall structure: ow triple-layered (owl1-3), bi-layered mw, and triple layered iw (iwl1-3); spore surface ornamentation of owl1, with dispersed, blunt papillae. **24**. Blunt ornamentation in planar view. **25–26**. mwl1 expanding in lactic acid based mountants; mwl2 with needle-tuberculate



ornamentation. Yellow brown to brown germination shields with germ tube initiations (gti). **27–30**. Germ shields (planar view) with 4–8 lobes, mostly bearing, each, one germ tube initiation (gti) from where germ tubes (gt) emerge; single lobes are separated by large folds (f), and a single germ pore (gp) connects the shield with the cell content. **31**. gti of the germ shield partly visible on MWL2.

Middle wall (MW) is hyaline to rarely light yellow, bi-layered and 5.2–7.6 (–8.9) µm thick when mounted in water. MWL1 is hyaline, and 4.0–5.1 µm thick in water. It expands to 7.5–14.6 µm in crushed spores and regularly expands further to 20–28(–51) µm in lactic acid based mountants. MWL2 is hyaline to light yellow, 1.2–2.5(–3.8) µm thick, and densely packed with small tuberculate-warty projections on the inner surface. Projections are 1.0–1.5(–2.5) µm long and 0.5–1.0(–1.1) µm broad. Warts are about (0.5–)1.0 (–2.5) µm apart from each other.

Inner wall (IW) is triple-layered bearing a germination shield on the outer surface. Outer layer of the inner wall (IWL1) is hyaline, semi-flexible and 0.5–1.2 µm thick. Second layer (IWL2) is unit, semi-flexible and 1.2–2.5 µm thick. Innermost layer (IWL3) is thin (0.4–0.8 µm thick), flexible, generally tightly adherent to IWL2, and difficult to observe. IWL2 stains reddish-brown to purple in Melzer's reagent.

Sporogenous cell is subglobose to elongate, concolorous with the spore, or slightly lighter in color than the spore, and 46–64 µm long and 71–76 (–79) µm broad. Two wall layers are generally visible on the sporogenous cell, continuous with OWL1 and OWL2. OWL1 on the sporogenous cell is about 0.5–1.6 µm; adherent OWL2 is about 2.5–7.6(–10.2) µm thick. The ornamentation on OWL1 does not continue on the wall of the sporogenous cell. The pore of the sporogenous cell is generally closed at the septum to the attached 'sporogenous hypha' by a septum arising from OWL2.

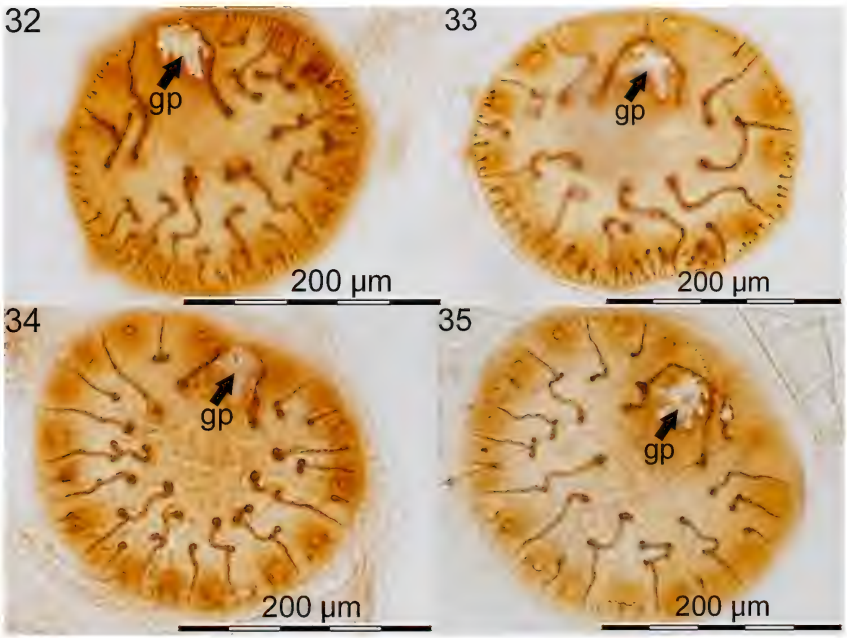
Germination shield is yellow-brown to brown, cardioid to oval or ellipsoid, 181–189 × 166–171 µm in diameter, and has several 4–8(–10) lobes, that are easy to differentiate as they are often wavy in planar view and separated by large folds. These folds (about 5–15 µm long) arise from the shield wall. The one-layered shield wall and the folds are generally only 0.5–1.7 µm thick. Each lobe regularly bears one rounded germ tube initiation, 1.2–2.5 µm in diameter, from where the germination tubes emerge.

Additional material examined: **BRAZIL. Rio Grande do Norte**, Natal, from sand dunes (URM 83483–83485).

Distribution: *Paradentiscutata maritima* has been recovered from two sand dune locations in NE Brazil (Mataraca, Paraíba State, and Natal, Rio Grande do Norte State).

Spore development in *Intraornatospora* and *Paradentiscutata*

In *I. intraornata* the outer spore wall is continuous with the wall of the sporogenous cell and the attached sporogenous hypha. The ornamentation on the inner surface of the outer spore wall differentiates during spore formation. The inner wall forms de novo during spore formation like an 'endospore'. In *Paradentiscutata* species, the outer wall is also continuous with the wall of the sporogenous cell and the sporogenous hypha attached. Although uncertain



Figs 32–35. *Dentiscutata biornata* (isotype, OSC 49583): Typical germination shields have one germ pore (gp) 12–24 compartments and 12–24 germ tube initiations (gti); compartments (dense lobes) separated by large folds; shield periphery is dentate.

whether the middle wall forms completely de novo or differentiates from a thin layer present on the inner surface of the outer wall, we did observe that during spore formation MWL1 differentiates first, showing several laminae when exposed to PVLG and other lactic acid based mountants. MWL2 differentiates on the inner surface of developed MWL1. Thereafter, IW is formed de novo, and, in the final stage of spore development the germination shield differentiates on the outer IW surface.

Discussion

Distinct spore morphologies were recognized for both *Paradentiscutata* and *Intraornatospora*. *Paradentiscutata* spp. have three-walled spores with a diagnostic tuberculate ornamentation on the inner surface of the middle wall and an openly organized, multiple-lobed, yellow-brown to brown germination shield on the outer IW surface. *Intraornatospora intraornata* has a tuberculate ornamentation of the inner OW surface, and on the outer IW surface a multiple lobed, hyaline to light yellow germination shield that regularly appears to be rudimentary even in older spores, since clearly visible germ tube initiations have rarely been found (Figs 2–4). Germination itself has yet to be observed in spores

of this species, so we do not know whether *I. intraornata* germinates through the rudimentary germ shield or directly from the warty inner projections of the outer wall (as in *Gigaspora*). The two new genera, morphologically somewhat dissimilar, might better be separated into two families. However, the database so far is too small to make a final conclusion on this aspect.

The two *Paradentiscutata* spp. can easily be distinguished through their ornamentation on the outer spore surface, a character that clearly separates species in other glomeromycete families (e.g., *Racocetra verrucosa* and *R. persica*, *Dentiscutata nigra* and *D. reticulata*, *Acaulospora scrobiculata* and *A. cavernata;* Oehl et al. 2008, 2011d). Of the *Gigasporales* species, *P. maritima* resembles only *Dentiscutata biornata*, which differs in a compact germination shield with multiple (12–24) compartments in mature spores (Figs 32–35) that are not openly organized or multiple-lobed (Spain et al. 1989) as in *P. maritima*, which has 4–8(–10) lobes. Additionally, the double ornamentation of *D. biornata* belongs to the outer spore wall according to Spain et al. (1989). These important morphological differences point out species dissemblance and support the new speciation. However, further research may consider checking the phylogenetic relationships between *D. biornata* and the two new species.

Species of *Intraornatosporaceae*, *Dentiscutataceae,* and *Gigasporaceae* are predominantly found in warmer climates. The *Intraornatosporaceae* have so far been detected only in tropical areas, while *Dentiscutataceae* spp. are known also from sub-tropical and Mediterranean regions and *Gigasporaceae* spp. have at times been recovered from temperate regions (Jansa et al. 2002, Oehl et al. 2010). It is possible that *Dentiscutataceae* and *Gigasporaceae* may have evolved to cooler periods and climates than *Intraornatosporaceae*. Further AM fungal diversity research will also help verify this hypothesis.

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The authors acknowledge, in special, the valuable comments and revisions of several experts on the manuscript and appreciate the corrections by Shaun Pennycook, Nomenclatural Editor, and suggestions by Lorelei L. Norvell, Editor-in-Chief. This work was supported by Protax (Program of Capacitation in Taxonomy), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), which provided a research grant to Leonor C. Maia and scholarships to Danielle K. Alves da Silva and Daniele M.A. de Assis; Programa de Biodiversidade do Semi-árido (PPBio); FACEPE (Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco) which provided financial support to G.A. Silva; Universidade Federal de Pernambuco (UFPE), which provided a grant to F. Oehl as 'visiting professor.' The authors would like to thank the Millennium Inorganic Chemicals Mining, a Cristal Company, for logistical support.

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Radulomycetopsis (*Agaricomycetes*), a new corticioid genus from India

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Abstract — A new corticioid genus, *Radulomycetopsis*, is described for the new species, *R. cystidiata*, from West Kameng in Arunachal Pradesh.

Key words — Eastern Himalaya, Bomdila, angiosperm host

While conducting a fungal foray in Bomdila in West Kameng of Arunachal Pradesh, India, Dhingra made a collection on decaying angiospermous branches. The macroscopic and microscopic characters were compared with similar genera in *Corticiaceae* (Rattan 1977, Thind & Dhingra 1985, Eriksson et al. 1981, Hjortstam et al. 1987) but could not be assigned to any already known, hence the description of a new genus. Morphological traits show similarities with *Radulomyces*.

Radulomycetopsis Dhingra, Priyanka & J. Kaur, **gen. nov.**

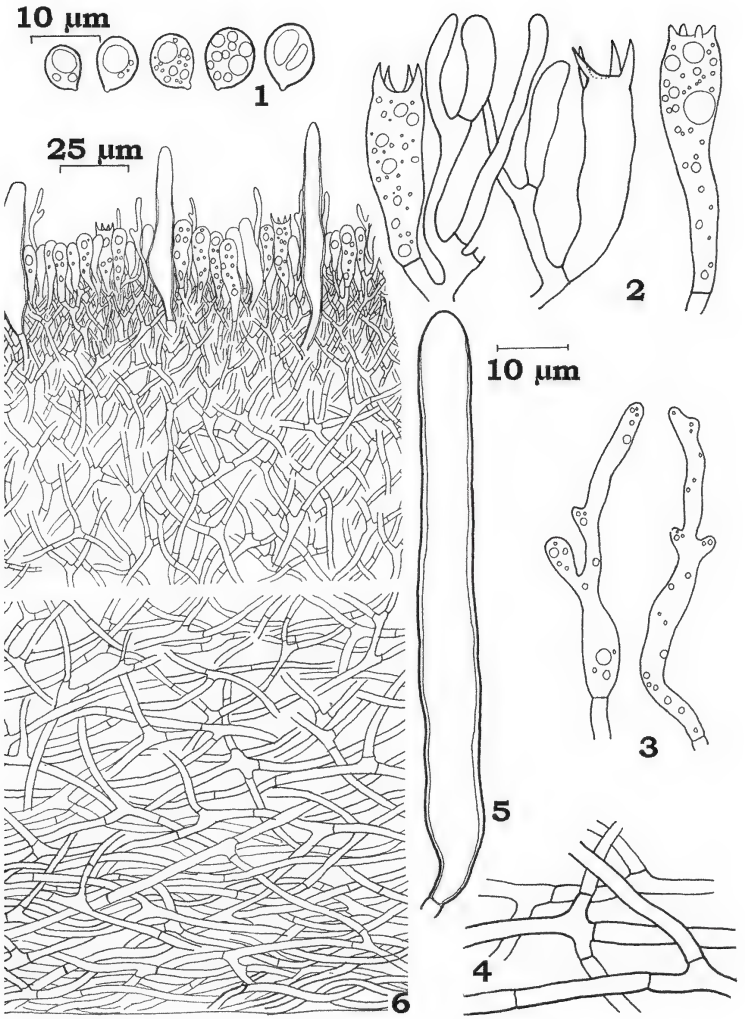
MycoBank MB 560517

Differs from *Radulomyces* in basidiocarps that are dark-colored, lack clamp connections, and possess cystidia.

Type Species: *Radulomycetopsis cystidiata* Dhingra, Priyanka & J. Kaur

Etymology: The name of the genus is based on the resemblance with genus *Radulomyces*.

Basidiocarp resupinate, adnate, effused, membranous-ceraceous; hymenial surface smooth to slightly tuberculate, orange to brownish orange to reddish brown; margins fibrillose, concolorous but paler; hyphal system monomitic; generative hyphae branched at wide angles, without clamps, basal hyphae covered with a thick sheath of brownish red matter, which dissolves in 3% KOH; cystidia projecting, thin- to slightly thick-walled; simple to somewhat branched hyphoid structures present in the hymenium; basidia clavate to



Figs 1–6. *Radulomycetopsis cystidiata*: microscopic structures.
1. Basidiospores; 2. basidia; 3. hyphoid structures in the hymenium; 4. generative hyphae; 5. cystidium; 6. vertical section through basidiocarp.

subclavate, 4-sterigmate, without a basal clamp; basidiospores broadly ellipsoid to subglobose, smooth, thin- to slightly thick-walled, inamyloid, acyanophilous; both basidia and basidiospores rich in oil drops.

Remarks— *Radulomycetopsis* resembles *Radulomyces* in producing simple to somewhat branched hyphoid structures in the hymenium, similar basidia,

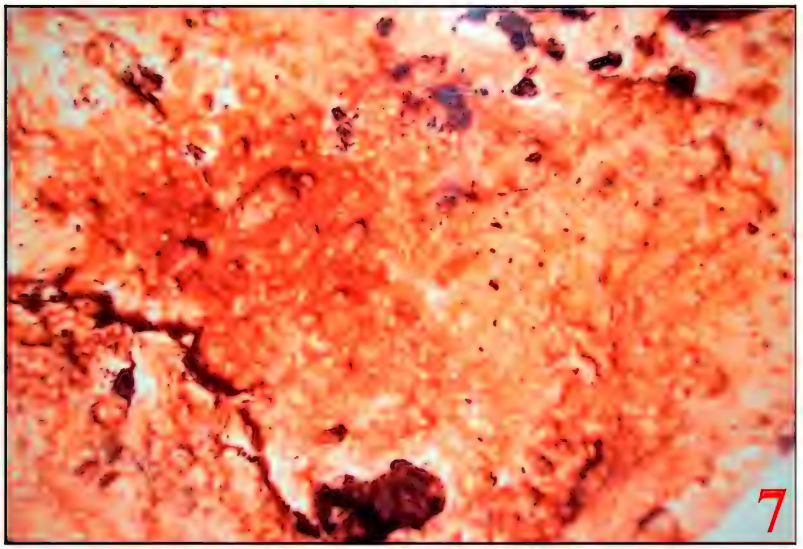


Fig. 7. *Radulomycetopsis cystidiata*: basidiocarp showing hymenial surface.

and broadly ellipsoid to subglobose basidiospores that are thin- to slightly thick-walled, non-amyloid, acyanophilous, and with oil rich protoplasm. *Radulomycetopsis* differs in having dark colored fruitbodies, hyphae without clamps, and presence of cystidia, a combination of features that supports an independent genus. A sample has been studied by Prof. Kurt Hjortstam, who supports the concept of a new genus.

Radulomycetopsis cystidiata Dhingra, Priyanka & J. Kaur, **sp. nov.**

MycoBank MB 560518 Figs 1–7

Differs from *Radulomyces confluens* by the absence of clamp connections and the presence of cystidia.

Type: India, Arunachal Pradesh: West Kameng, Bombila, on decaying angiospermous branches, 29 August 1981, Dhingra 19767 (PAN, **holotype**).

Etymology: The epithet refers to the presence of cystidia.

Basidiocarp resupinate, adnate, effused, up to 430 µm thick in section, membranous-ceraceous; hymenial surface smooth to slightly tuberculate, orange to brownish orange to reddish brown when fresh, becoming orange gray to grayish orange to brownish orange on drying; margins thinning, irregular in outline, fibrillose. Hyphal system monomitic; generative hyphae up to 3 µm wide, thin-walled, branched at wide angles, septate, without clamps; basal zone composed of sparsely branched hyphae, running almost parallel to the substrate, covered with some brownish-red matter which dissolves in 3%

KOH, followed by a zone of comparatively more richly branched, loosely and irregularly interwoven hyphae which merge into the hymenial zone where the hyphae become vertical and much branched forming a dense texture. Cystidia 70.0–85.0 × 9.0–11.0 µm, cylindrical to sub cylindrical, thin- to somewhat thick-walled, projecting up to 50 µm out of the hymenium. Some simple to slightly branched hyphoid structures, are also observed in the hymenium. These are thin-walled, with oily contents, and look different from dendrohyphidia. Basidia 25.0–37.0 × 7.0–8.5 µm, clavate to subclavate, often constricted with oily contents, 4-sterigmate, with no basal clamp; sterigmata up to 7.0 µm long. Basidiospores 6.0–9.0 × 5–7.25 µm, broadly ellipsoid to subglobose, thin- to slightly thick-walled, inamyloid, acyanophilous, uniguttulate or with many oil drops.

Remarks—*Radulomycetopsis cystidiata* is similar to *Radulomyces confluens*, which is easily distinguished by the presence of clamp connections and absence of cystidia.

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First record of *Dendrothele mangiferae* (*Agaricales, Basidiomycota*) from the Neotropics

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Abstract — *Dendrothele mangiferae*, previously known only from the Mascarene Islands, is reported for the first time from Central America. It has been found in Costa Rica growing on bark of living *Terminalia catappa*. A full description, line drawing, and colour photos are provided.

Key words — corticioid fungi, Manuel Antonio, ornamented basidiospores

Introduction

A conspicuous corticioid species, *Dendrothele mangiferae*, has recently been collected from Costa Rica on bark of living *Terminalia catappa* L. (*Combretaceae*), a large tree naturally widespread in subtropical and tropical zones of Indian and Pacific Oceans and planted extensively throughout the tropics. It constitutes the first record from outside the Mascarene Islands of Réunion and Mauritius (Boidin et al. 1996). Specimens are fully described and illustrated. Additional comments on the generic placement and related species are provided.

Materials & methods

For light microscopic studies, samples were mounted in 3% potassium hydroxide (KOH), Melzer's reagent (IKI), and 0.1% cotton blue in 60% lactic acid to determine cyanophily of basidiospore walls. Line drawings were made with a camera lucida attachment. Specimens are deposited in INB and SALA.

Taxonomy

Dendrothele mangiferae Boidin & Duhem, Bull. Soc. Mycol. France 112(2): 106, 1996. Plates 1–2

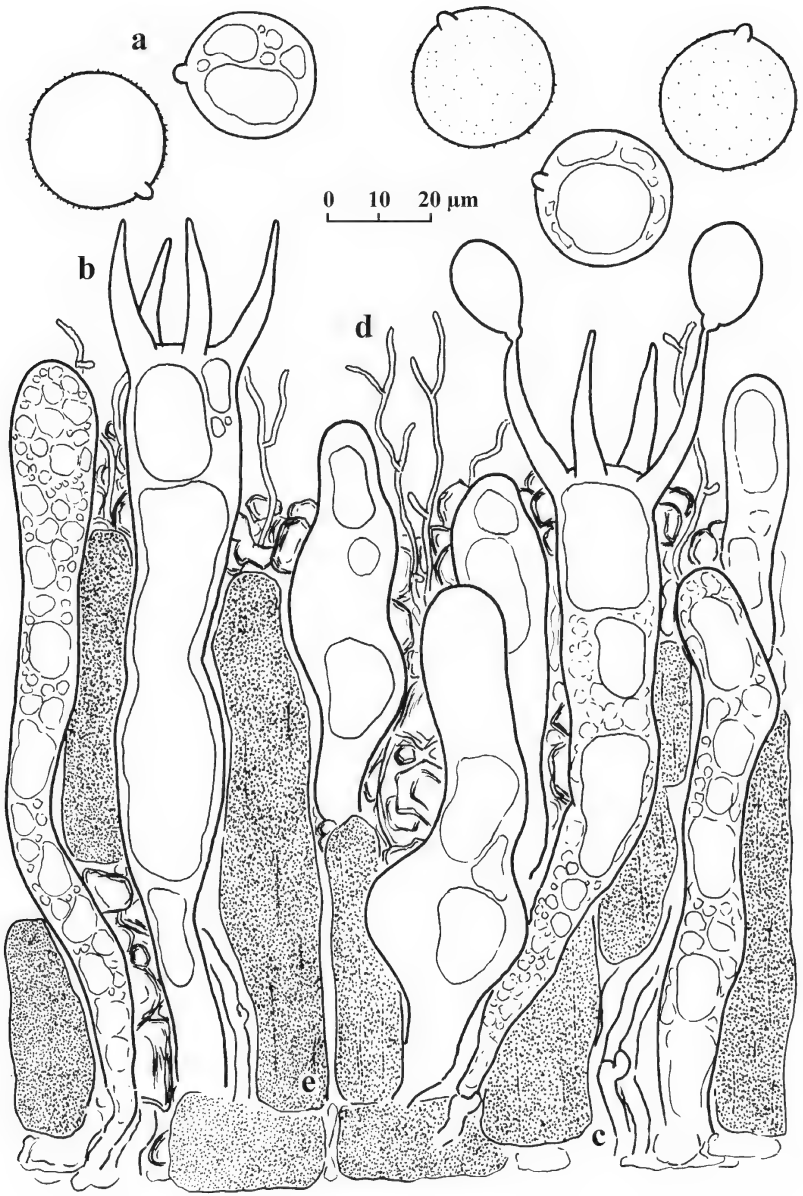

Plate 1. *Dendrothele mangiferae* (S.P. Gorjón 3383).
Hymenial elements: a) basidiospores; b) basidia; c) hyphae; d) dendrohyphidia; e) host cells.

PLATE 2. *Dendrothele mangiferae* (S.P.Gorjón 3383). Basidiome. Scale Bar = 2 cm.

BASIDIOMATA resupinate, orbicular at first, then effuse, adnate, white, hymenial surface smooth, cracked when mature, margin abrupt. HYPHAL SYSTEM monomitic, hyphae clamped, 2–4 µm wide, thin-walled, obscured by the abundant crystalline encrustation. DENDROHYPHIDIA filamentous, sinuous, with few branches, clamped. BASIDIA suburniform at first, then subcylindrical, cyanophilous, 100–150 × 15–25 µm, basally clamped, with 4 long sterigmata about 15–25 µm. CYSTIDIA absent. BASIDIOSPORES subglobose, (22–)24–27 × 22–25 µm, when immature and still attached to the sterigmata broadly ellipsoid, seemingly smooth but minutely spinulose in Melzer's reagent and cotton blue, walls hyaline, slightly thickened, with a conspicuous blunt apiculus, faintly cyanophilous, inamyloid, nondextrinoid.

HABITAT AND DISTRIBUTION — Known from the Mascarene Islands growing on trunks of *Mangifera indica* L. (*Anacardiaceae*) and undetermined trees; and from Costa Rica on bark of living *Terminalia catappa* on the Pacific coastal shore (PLATE 3).

SPECIMENS EXAMINED — **COSTA RICA. MANUEL ANTONIO**, Espadilla beach, 9°23′35″N 84°09′12″W, 2–5 m a.s.l., 23.VII.2011, on bark of living *Terminalia catappa*, leg. S.P. Gorjón, coll. 3380, 3381, 3382, 3383, 3384.

COMMENTS — *Dendrothele mangiferae* is easily recognized by the large globose and minutely ornamented basidiospores. Within *Dendrothele* Höhn. & Litsch.,

PLATE 3. Habitat of *Dendrothele mangiferae* with *Terminalia catappa* trees on the Pacific coast of Costa Rica.

there are two additional species with spinulose basidiospores: *Dendrothele candida* (Schwein.) P.A. Lemke, with globose basidiospores (13–)15–20 × (11–)13–16(–20) µm and clampless hyphae; and *Dendrothele nivosa* (Berk. & M.A. Curtis ex Höhn. & Litsch.) P.A. Lemke, with subglobose to ovoid basidiospores 16–24 × 14–17 µm and clamped hyphae. Phylogenetic studies show that *Dendrothele* is a polyphyletic genus, and that *D. nivosa* seems not to be closely related to other *Dendrothele* species, including the generic type, *D. papillosa* Höhn. & Litsch. [= *D. griseocana* (Bres.) Bourdot & Galzin] (Karen K. Nakasone, pers. comm.). Further studies are needed within *Dendrothele* species with ornamented basidiospores to elucidate their phylogenetic relationships.

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Two new fungi from Mexico: *Anaseptoidium* gen. nov. and *Cylindrosympodium sosae* sp. nov.

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Abstract — Two new anamorphic fungi are described and illustrated from a cloud forest in Mexico. *Anaseptoidium mycophilum* gen. & sp. nov. is distinguished by monoblastic, integrated, mostly determinate conidiogenous cells and solitary, 2–3-septate, oblong to cylindrical, pale brown conidia that usually remain attached after maturity. *Cylindrosympodium sosae* sp. nov. is characterized by cylindrical, mostly 4–5-septate, smooth, hyaline to subhyaline, sometimes guttulate conidia.

Key words — mycophilous fungi, systematics

During an investigation of microfungi on dead plant material in a cloud forest located in the private protected area "Agüita Fria", Veracruz, Mexico, two undescribed anamorphic fungi were collected. Individual samples of plant material were placed in paper and plastic bags taken to the laboratory and treated according to Castañeda (2005). Mounts were prepared in polyvinyl alcohol-glycerol (8 g in 100 ml of water, plus 5 ml of glycerol) and measurements made at a magnification of ×1000. Micrographs were obtained with a Nikon Eclipse E600 light microscope (Japan). One new genus and two new species are described below.

Taxonomy

Anaseptoidium R.F. Castañeda, Heredia & R.M. Arias, anam. gen. nov.
MycoBank MB561820

Coloniae in substrato naturali effusae, reptantes, funiculosae, brunneae. Stomatopodia absentia. Conidiophora macronematosa interdum in cellulla conidiogenae reducta. Cellulae conidiogenae monoblasticae, determinatae. Conidia solitaria, acrogena, cylindrica usque ad oblonga, fimbriate, septata, pallide brunnea ad brunnea.

Type species: *Anaseptoidium mycophilum* R.F. Castañeda et al.

Etymology: Greek, *Ana-*, meaning upwards, back, and again; Latin, *septoidium*, referring to an anamorphic genus *Septoidium*.

Colonies on the natural substrate effuse, creeping, funiculose, brown. Mycelium superficial and immersed. Stomatopodia absent. Conidiophores macronematous, mononematous, erect, sometimes reduced to conidiogenous cells. Conidiogenous cells monoblastic, integrated, determinate. Conidial secession schizolytic. Conidia solitary, acrogenous, cylindrical to oblong, fimbriate, septate, smooth or verruculose, pale brown to brown. Teleomorph unknown.

Anaseptoidium mycophilum R.F. Castañeda, Heredia & R.M. Arias, **anam. sp. nov.** Figs. 1, 2

MycoBank MB561823

Conidiophora macronematosa, 1–2-septata, 7.6–17.3 × 4.5–5.5 µm, interdum in cellula conidiogena reducta. Cellulae conidiogenae monoblasticae, 4–17 × 2–6.5 µm. Conidia acrogena, oblonga vel cylindrica, (1–)2(–3)-septata, plerumque eccentrica fimbriata ad basim, 12–17 × 4–6 µm.

Type: Mexico, Veracruz: Agüita Fria, 19°31′N 96°59′W, on synnemata of *Phaeoisaria clavulata* (Grove) E.W. Mason & S. Hughes, 8 February 2010, G. Heredia & V. Sosa (**Holotype**: XAL CB1696).

Etymology: Greek, *myco-*, meaning fungus, fungal, Greek, *-philum*, meaning loving.

Colonies on the natural substrate, effuse, creeping, funiculose, brown, mycophilic. Mycelium mostly superficial. Hyphae septate, branched, 2–4 µm diam., smooth, pale brown, ascending on the synnematal conidiomata of the host. Stomatopodia absent. Conidiophores macronematous, mononematous, erect or prostrate, straight, simple, 1–2-septate, smooth, 7.6–17.3 × 4.5–5.5 µm, very pale brown, sometimes reduced to conidiogenous cells. Conidiogenous cells monoblastic, terminal, determinate, integrated, pale brown, 4–17 × 2–6.5 µm. Conidial secession schizolytic. Conidia solitary, acrogenous, oblong to cylindrical, (1–)2(–3)-septate, mostly eccentric and fimbriate at the base, rounded at the apex, smooth, 12–17 × 4–6 µm, very pale brown, dry, usually remaining attached to the conidiogenous loci after maturity. Teleomorph unknown.

Note: *Anaseptoidium* resembles *Septoidium* G. Arnaud, which differs in monoblastic, percurrently proliferating, annellate conidiogenous cells and stomatopodia formed regularly by the assimilative hyphae on the substrate. Most species are plant pathogenic (Baker 1955, Ellis 1971, 1976). Esquivel

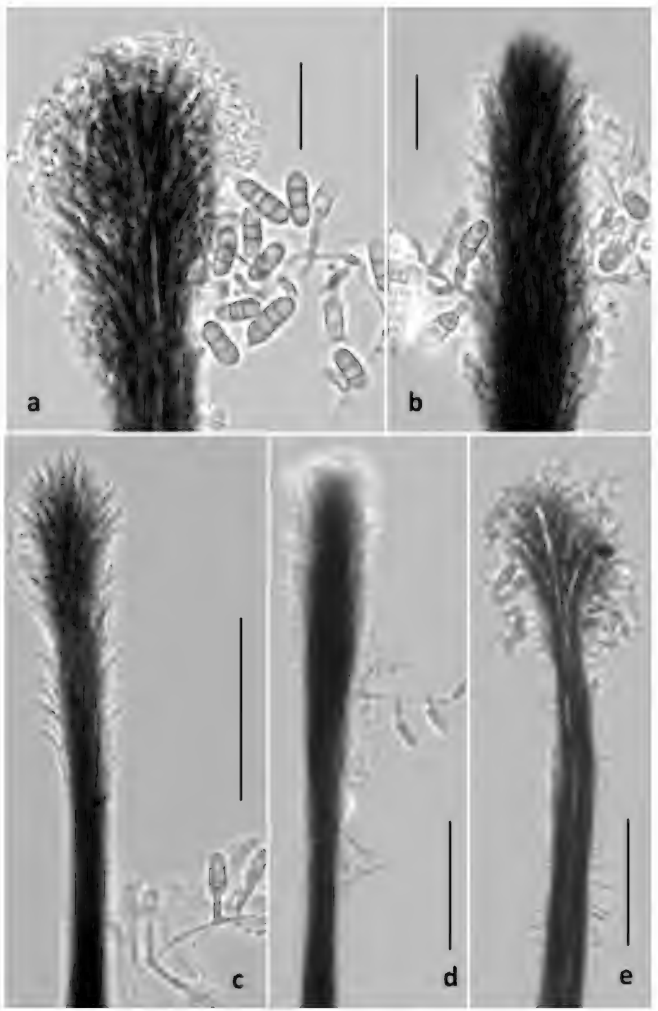

FIG. 1. *Anaseptoidium mycophilum* (ex holotype XAL CB1696, on synnemata of *Phaeoisaria clavulata*). a–e. Conidiophores, conidiogenous cell and conidia. a–b. Scale bars = 20 μm, c–e scale bars = 50 μm.

(2011), who illustrated several conidiogenous events observed in the *Septoidium* anamorph of *Parodiopsis hurae* (G. Arnaud) R.E.D. Baker & W.T. Dale, confirmed the enteroblastic percurrent proliferations of the conidiogenous cells in *Septoidium*.

In *Anaseptoidium,* the secession of each conidium is schizolytic, but the outer and inner wall layers do not separate simultaneously as in most related fungi. The outer wall layer(s) breaks first, and many conidia are observed attached

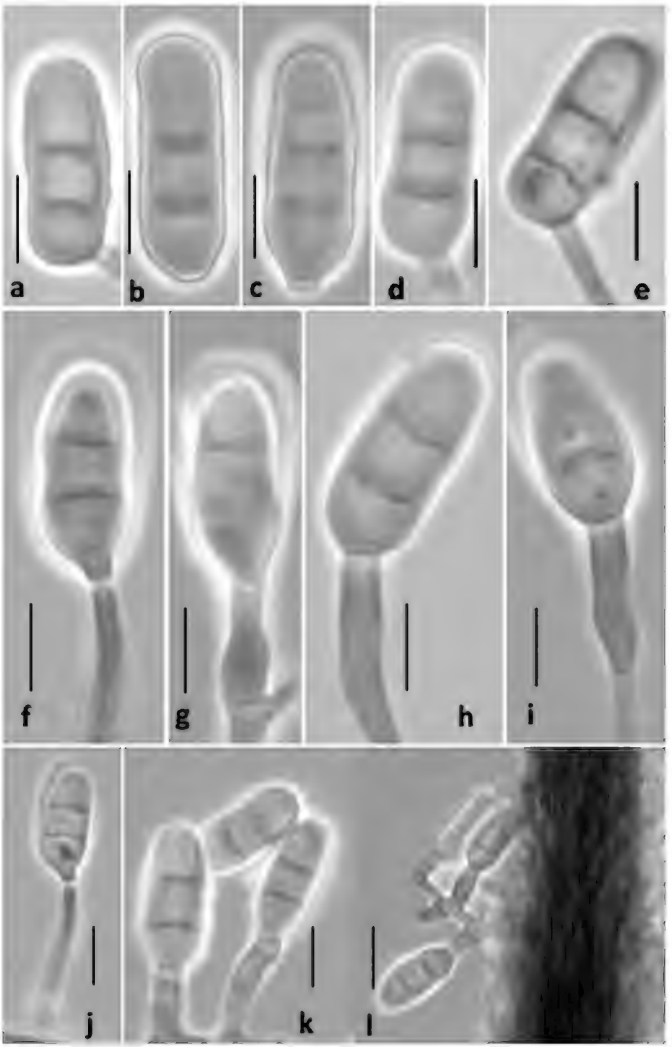

Fig. 2. *Anaseptoidium mycophilum* (ex holotype XAL CB1696). a–i. Conidiogenous cells and conidia. Scale bars = 5 μm, j–l. Conidiophores, conidiogenous cells and conidia. Scale bars = 10 μm.

only by the inner wall layer(s) as can be confirmed by the pale or colorless pigmentation of the conidiogenous cell apex just below each attached conidium (Fig. 2 a–k). The inner wall layer(s) may separate independently of the outer wall separation(s). As a result, conidia are seen somewhat separated (1–2 μm) from the outer wall layers while still attached on each conidiogenous locus.

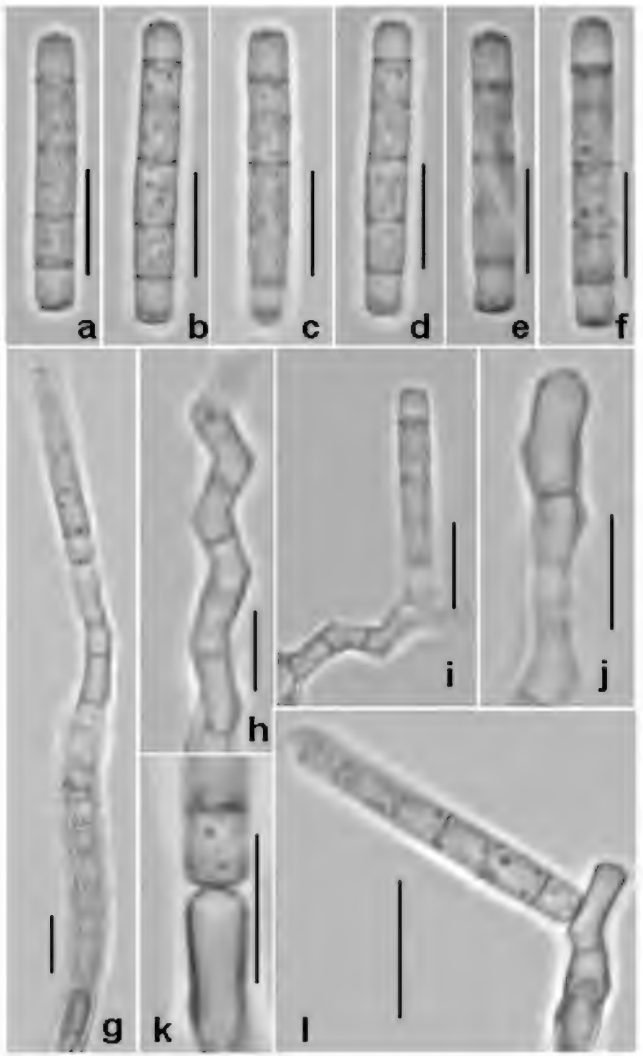

Fig. 3. *Cylindrosympodium sosae* (ex holotype XAL CB1699). a–f. Conidia, g. Conidiophore, conidiogenous cells and conidium. h–l. Conidiogenous cells and conidia. Scale bars = 10 μm.

Cylindrosympodium sosae R.F. Castañeda, Heredia & R.M. Arias, **anam. sp. nov.** Figs. 3, 4

MycoBank MB561822

Ad Cylindrosympodii africani *differt in conidiophora macronematosa, 6–15-septata, 71–106 × 5–10 μm, leprosa vel verruculosa ad basim et conidia 4–5-septata, 25.5–28.5 × 4–4.5 μm.*

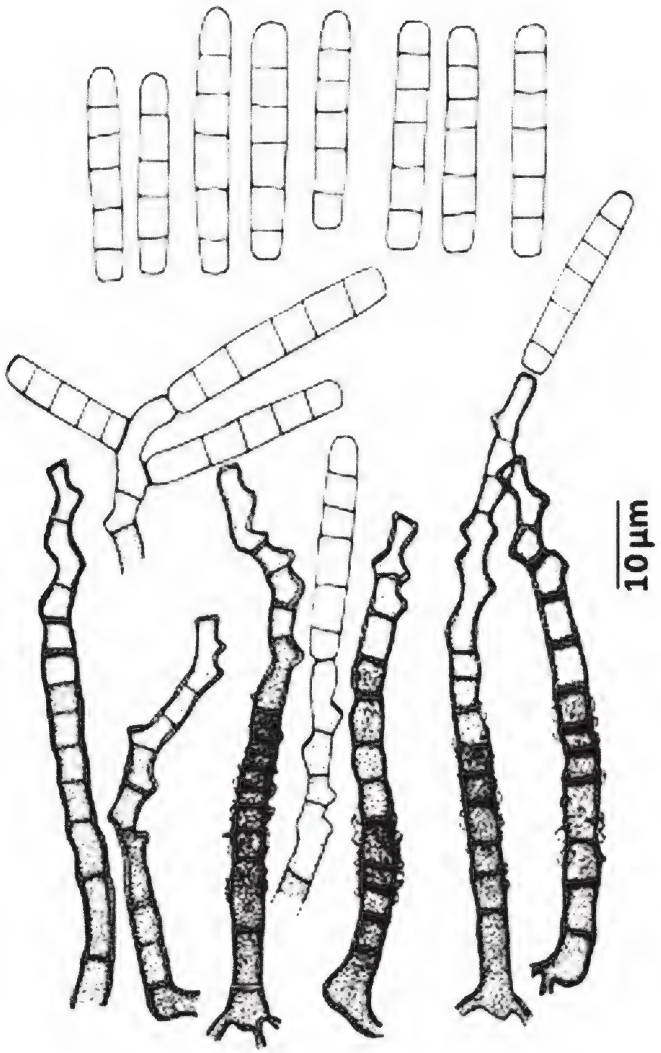

Fig. 4. *Cylindrosympodium sosae* (ex holotype XAL CB1699). Conidiophores, conidiogenous cells and conidia.

Type: Mexico. Veracruz: Agüita Fria, 19°31′N 96°59′W, on decaying leaf of *Liquidambar styraciflua* L. (*Hamamelidaceae*), 8 February 2010, V. Sosa (**Holotype**: XAL CB1699).

Etymology: Latin, *sosae*, in honor to Dr. Vinicio Sosa Fernández (INECOL, Mexico) who collected and kindly provided the specimen.

Colonies on the natural substrate effuse, pilose, yellowish brown or reddish brown. Mycelium mostly immersed. Hyphae septate, branched, smooth or verruculose, yellowish brown or brown, 1.5–3 µm diam. Conidiophores macronematous, mononematous, erect, straight or flexuous, simple, 6–15-septate, verruculose to leprous near the base and smooth towards the apex, 71–106 × 5–10 µm, yellowish brown or reddish brown below, hyaline or subhyaline towards the apex. Conidiogenous cells polyblastic, integrated, intercalary and terminal, subhyaline or hyaline, 8–15 × 3–4.5 µm, geniculate, indeterminate, with several holoblastic sympodial proliferations and flattened conidiogenous loci. Conidial secession schizolytic. Conidia solitary, acropleurogenous, cylindrical, truncate at the base, 4–5-septate, smooth, hyaline or subhyaline, 25.5–28.5 × 4–4.5 µm, sometimes guttulate, dry. Teleomorph unknown.

Note: *Cylindrosympodium* is distinguished by "flat-topped denticles" on conidiogenous cells that form solitary, cylindrical, septate, hyaline conidia (Castañeda-Ruiz & Kendrick 1990, 1991, Paulus et al. 2003, Marvanová & Laichmanová 2007, Crous et al. 2007). *Cylindrosympodium sosae* strongly resembles *C. africanum* (Morgan-Jones et al.) Marvanová (Marvanová & Laichmanová 2007), which is easily differentiated by its brown or pale brown smooth conidiophores and 3–4-septate 31–33 × 2 µm conidia (Morgan-Jones et al. 1983).

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Candelariella, Ochrolechia, Physcia, and *Xanthoria* species new to Turkey

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Abstract — *Candelariella kuusamoensis, Ochrolechia frigida, Physcia phaea,* and *Xanthoria papillifera* were determined as new to Turkey and the Middle East following a recent lichenological foray in Iğdır and Van regions (Turkey). Geographic distribution, substrate, chemistry, and comparisons with morphologically similar taxa are presented.

Key words — *Ascomycetes*, biodiversity, lichen

Introduction

Although many lichen taxa have recently been recorded for Turkey (Candan et al. 2010, Candan & Halici 2009, Halici et al. 2010a,b, Kinalioglu 2009, Kinalioğlu & Aptroot 2010, Kinalioglu 2010a,b,c, Karagöz et al. 2011, Perez-Ortega et al. 2010, Yazici & Aslan 2009, 2010a,b), the lichen biota of many regions are still unknown. No lichenized fungi have thus far been reported for the Iğdır region, although some surveys of lichens have been conducted in the neighbouring Ardahan region (Osyczka et al. 2011, Yazici et al. 2010a,b) and 81 taxa were reported from the Van province (Aslan & Öztürk 1995, Szatala 1941, 1960, Şenkardeşler & Lökös 2010). Ten *Candelariella*, fifteen *Ochrolechia*, sixteen *Physcia,* and twelve *Xanthoria* taxa have previously been reported from Turkey.

The climate of the Iğdır region is characterized by hot dry summers and cold snowy winters. The mean annual temperature is 11.6°C, average humidity is 63%, and the mean annual rainfall is about 257.6 mm. Summers are characterized by moderate precipitation and the winters are generally marked with high precipitation (Akman 1999).

The Iğdır region has few forested areas and is dominated by steppe. Mountain Zordağ (Iğdır: Center), which is exposed to high light conditions, is a windswept

area with gently sloping terrain dominated by grass, rocks, and streams with scattered coniferous and deciduous trees. *Prunus*, *Pyrus*, *Populus*, *Salix*, and *Elaeagnus* trees are seen occasionally along the stream between Taşköprü and Karaca villages (Tuzluca) (Baytop & Denizci 1963). There are no trees but many calcareous and siliceous rocks in the Taşucan and Bahçecik villages, an exposed windswept area.

The Van region has a continental climate, characterized by long cold snowy winters and hot dry summers. The mean annual temperature is 9°C, average humidity is 51.10% , and the mean annual rainfall is about 380 mm, with a precipitation range of 370–570 mm (Akman 1999). The collection sites are exposed to high light conditions and windswept with gently sloping terrain comprising grass and volcanic calcareous rocks. A river valley with trees was sampled only in Saray town (Van). In the other regions of the Van district there are no deciduous or coniferous trees (Baytop & Denizci 1963).

Material & methods

During a recent lichenological research foray in Iğdır and Van regions (Turkey), lichen samples were collected from 07 December 2008 to 27 September 2009 and 13 June 2010 to 12 July 2010. Air-dried samples were examined with a Nikon SMZ1500 stereomicroscope and a Nikon Eclipse 80i light microscope. Identifications were determined by consulting appropriate keys in the literature (Brodo et al. 2001, Dobson 2005, Kärnefelt 1990, Kukwa 2009, Moberg 1977, Poelt 1974, Poelt & Vězda 1981, Redchenko et al. 2010, Smith et al. 2009, Thomson 1997, Wetmore 2005, Wirth 1995). Secondary metabolites were identified by the usual spot tests.

Vouchers are stored in the herbarium of the Biology Department, Science Faculty, Karadeniz Technical University, Trabzon, Turkey (KTUB) and the Biology Department, Kazım Karabekir Education Faculty, Atatürk University, Erzurum, Turkey (ATA-KKEF).

Species recorded

Candelariella kuusamoensis Räsänen, Ann. Bot. Soc. Zool.-Bot. Fenn. Vanamo, 12(1): 56.1939 Fig 1

Thallus muscicolous, thick, dense, granular-warty, yellow to orange-yellow, to 3.2 cm in diam., almost cushion or aggregated break parts-like, to deep areolate cracks, consisting of closely crowded large granules (0.1–0.7 mm), upper surface uneven, yellow. Medulla green. Apothecia rare, to 0.25–1mm diam., asci clavate, *Candelaria*-type, 12–32-spored, ascospores 9–15 × 4.5–5.5 µm, non-septate, hyaline. Algae trebouxioid. Hypothecium hyaline; hymenium hyaline, *c.* 65–70 µm high. Paraphyses 2.5–3µm thick. Cortex K –, C –, KC –, P –. Medulla K–, C–, KC–, P –. Thallus contains pulvinic acid.

Candelariella kuusamoensis grows on moss, plant remains, soil, more rarely on rocks and wood and wooden fences, and on the top of poles in alpine areas.

Fig. 1. *Candelariella kuusamoensis,* habitus. Scale= 1mm

Previously known from Asia, Europe, and North America. New to Turkey and the Middle East.

Specimen examined: **TURKEY. Iğdır:** Center, Zordağ mountain, 39°45′49.05″N 43°53′20.13″E, on mosses, 2400m, 13.06.2010, (leg. K.Yazici,) KTUB–2023.

Ochrolechia frigida (Sw.) Lynge, Lich. Nov. Zemlya: 182.1928 Fig 2

Thallus crustose, muscicolous, to 1.2–2 cm diam., ± orbicular, thin crust or thick granules, effuse, ± verrucose, uneven-granulate, occasionally with few cylindrical-coralloid and branching spinulose extensions, whitish to yellow-white, pinkish or gray, soralia rare. Apothecia rare, to 1 mm wide, sessile to adnate; margin thin to slightly thickened, entire or wrinkled; disk flat, red-brown, slightly roughened, epruinose. Hypothecium dull brown; epihymenium brown; hymenium 130–180 µm, hyaline; paraphyses slender; Asci 8-spored, ascospores simple, ovoid, 26–43 × 15–26 µm. Cortex K–, C+ red, KC+ red, P–; medulla K–, C+ red, P–; disc K–, C+ pink to red, KC + red, P–. Thallus contains gyrophoric acid, ± variolaric acid.

Ochrolechia frigida is a bipolar lichen, occuring mainly on soil, mosses, humus, plant remains, and at the base of plants, It is especially found in raised bogs on mountain tops in arctic-boreal and alpine regions in the northern

Fig. 2. *Ochrolechia frigida*, habitus. Scale= 1cm

hemisphere. Previously known from Antarctica, Asia, Australasia, Europe, North America, and South America. New to Turkey and the Middle East.

Specimen examined: **TURKEY. Iğdır:** Tuzluca, between Taşköprü-Karaca villages, 39°52′07.40″N 43°28′57.94″E, 2134m, 14.6.2010, (leg. K.Yazici,) KTUB–2038.

Remarks— *Ochrolechia frigida* differs from *O. upsaliensis* and *O. alaskana* in its smaller ascospores (25–40 × 15–26 µm) and apothecia (*O. upsaliensis* has ascospores 55–75 × 26–38 µm). Also, *O. alaskana* has variolaric acid, which *O. frigida* lacks (Kukwa 2009). In addition, the thallus of *O. frigida* is delicate, grey, brown-grey, or brown, occasionally with a yellow tinge and distinct, branched spines, while the thallus in *O. alaskana* is thick and firm with a yellow tinge, and spines are lacking or occur occasionally in some specimens.

Physcia phaea (Tuck.) J.W. Thomson, Beih. Nova Hedwigia 7: 54. 1963 Fig 3

Thallus ± orbicular, or irregular, to 1.5–2.2 cm in diam., usually closely appressed to the substratum, steel-gray to dark gray, or brownish gray, white maculae present, rugose; pruina, soredia, isidia or lobules absent; lobes radiating, flat to slightly convex, 1–1.5 mm wide, typically crowded and overlapping, eciliate. Medulla white. Lower surface white to dark gray or brown, with white to black rhizines. Lower surface pale tan to brownish, with abundant

Fig. 3. *Physcia phaea*, habitus. Scale= 1mm

or sparse pale to brown rhizines, 0.3–1mm long. Apothecia numerous, 0.5–1.5 mm diam., margins lecanorine, crenulated when young, perfectly round and somewhat raised; disc dark brown to black, rarely covered with a white pruina; ascospores brown, 1-septate, *Physcia*-type, 16–20 × 7–9 µm. Cortex K + yellow, C + slightly yellow, KC + slightly yellow, P –. Medulla K + yellow, C –, KC ± yellow, P –. Thallus contains atranorin and zeorin. Pycnidia numerous; conidia immersed, subcylindrical, 4–6 × 1 µm.

Physcia phaea occurs mainly on granitic (less commonly, calcareous) rocks, or mosses (mostly in open areas), also on basaltic or gneissic and nutrient-enriched rocks. Previously known from Asia, Australasia, Europe, and North America. New to Turkey and the Middle East.

Specimens examined: **TURKEY. Iğdır:** Tuzluca, Taşucan village, 39°51′26.61″N 43°35′41.06″E, on calcareous rock, 3168m, 02.07.2010, (leg. K.Yazici), KTUB–2039. **Van:** Başkale, Başkale-Aşağı Dikmen Yol Ayrımı, Çamlık Köprü Mevkisi: 37°57′09″N 44°05′35″E, on calcareous rock, 1886 m, 29.04.2009, (leg. A.Aslan), ATA-KKEF-1951; Gevaş, Akdamar Adası Karşısı, Akdamar Camping Yanı, 38°18′32″N 43°02′24″E, on calcareous rock, 1662 m, 05.09.2009, (leg. A.Aslan), ATA-KKEF-1955; Center, Gedikbulak Köyü, Tuzla Karşıs, 38°51′13″N 43°26′32″E, on calcareous rock, 1723 m, 07.09.2009, (leg. A.Aslan), ATA-KKEF-1954, Van Kalesi, 38°30′14″N 43°19′56″E, on calcareous rock, 1681 m, 07-11.12.2008, (leg. A.Aslan), ATA-KKEF-1953, Erek Dağı Doğu Yamacı: 38°29′59”N 43°29′22″E, on calcareous rock, 1976 m, 01-05.10.2008, (leg.

A.Aslan), ATA-KKEF-1950; Çaldıran, Çaldıran Çıkışı, 39°08′07″N 43°55′45″E, on calcareous rock, 2053 m, 28.04.2009, (leg. A.Aslan), ATA-KKEF-1952.

Remarks— *Physcia phaea* resembles *P. aipolia* and *P. cascadensis,* but is distinguished from *P. aipolia* by occasionally growing on a bark and having apothecia that lack soralia and the heavy pruina characteristic of *P. aipolia. Physcia cascadensis,* a rare and rock-dwelling rosette lichen, like *P. phaea* lacks soredia and has a K + yellow medulla but differs in its very pale gray cortex, absence of maculae, and pruinose apothecia.

Fig. 4. *Xanthoria papillifera,* habitus. Scale= 1mm

Xanthoria papillifera (Vain.) Poelt, Mitt. Bot. Staatss. München 2: 29.1954 Fig 4

Thallus crustose, small, to 5–15 mm diam., forming ± appressed, ± orbicular, rosettes, reddish-orange, orange-yellowish; lobes well-developed, inflated to flattened, or pulvinate, 0.4–0.6 mm wide, 1.5–1.8 mm long, separate or ± overlapped high-arched, , curved, ± deeply lobed, mostly clearly separated. Soredia scattered and isidia-like; isidia dense mostly central regions of the thallus, coralloid or papillate, finger-like, pruinose, uniformly thick. Apothecia rare, lecanorine 0.2–0.5 mm diam. Asci 8-spored, ascospore 1-septate, colourless. Cortex on upper and lower thallus with hyphae ± perpendicularly oriented to the upper surface, attached by rhizines or adhering discs. Cortex K + purple, C + orange turning orange-brown, K + orange-brown, P –. Medulla K –, C –, KC –, P–. Apothecia K + purple. Thallus contains anthraquinones.

Xanthoria papillifera grows on steep surfaces of calcareous and siliceous rocks, boulders, and mossy soil, often in montane areas. Previously known from Africa, Asia, Australasia, Europe, and North America. New to Turkey.

Specimens examined: **TURKEY. Iğdır:** Center, Zordağ mountain, 39°45′49.05″N 43°53′20.13″E, on calcareous rock, 2400m, 13.06.2010, (leg. K.Yazici) KTUB–2022; Tuzluca, Bahçecik village, on roadside, 40°04′05.63″N 43°25′35.47″E, on calcareous rock, 1754m, 12.07.2010, (leg. K.Yazici), KTUB–2021. **Van:** Saray, Saray-Kapıköy Yolu Kaz Gölü Civarı, 38°32′06″N 44°15′21″E, on calcareous rock, 2180 m, 27.09.2009, (leg. A.Aslan), ATA-KKEF-1957; Çaldıran, Tendürek Dağı, Uzunyol Köyü, 39°19′08″N 44°01′56″E, on calcareous rock, 2351 m, 27.04.2009, (leg. A.Aslan), ATA-KKEF-1956.

Remarks— *Xanthoria papillifera* is similar to *X. sorediata,* which is distinguished by rounded to flattened lobes and sorediate isidia. This species has also been called *Xanthoria domogledensis* Vězda.

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A new species and new combinations and records of *Hypotrachyna* and *Remototrachyna* from Bolivia

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Abstract — *Remototrachyna sipmaniana* is described as new to science, and three new combinations, *R. aguirrei*, *R. consimilis*, and *R. singularis*, are proposed. Ten *Hypotrachyna* and two *Remototrachyna* species are reported as new to Bolivia, including the southernmost localities of *H. halei* and *H. partita*, the first record of *H. primitiva* from the southern hemisphere, and the second locality for *H. neoscytodes*.

Key words — foliose lichens, Neotropics, *Parmeliaceae*, South America

Introduction

Parmeliaceae Zenker (*Lecanorales*, *Lecanoromycetes*) is a large family of *Ascomycota* consisting of foliose, fruticose or rarely crustose lichens, but also lichenicolous non-lichenized taxa (e.g. Elix 1993, Lumbsch & Huhndorf 2007, Peršoh & Rambold 2002). It is believed to be one of the richest in species within the phylum and comprises about 1500 species (Blanco et al. 2006). This group has been extensively investigated by several researchers for more than 50 years, so the worldwide distribution of many taxa is well known. However, some areas, especially in the tropics, need further study; one of these is Bolivia. Data on *Parmeliaceae* from this country are not numerous and are mostly included in monographic treatments (e.g. Hale 1965, 1975, 1976; Culberson & Culberson 1981, Nash et al. 1995, Sipman et al. 2009) or rarely floristic papers (e.g. Herzog 1922, Feuerer & Sipman 2005).

During several lichenological expeditions to Bolivia we have collected more than 20,000 specimens and, in addition, further samples have been obtained on loan from several herbaria. Among the macrolichens, a large proportion were representatives of *Parmeliaceae*. This is the first paper dealing with new distributional data for *Parmeliaceae* in Bolivia. Here we report a new species and records for two morphologically similar, but phylogenetically distant genera, *Hypotrachyna* (Vain.) Hale and *Remototrachyna* Divakar & A. Crespo. Additionally, we propose transferring three additional species from *Hypotrachyna* to *Remototrachyna* based on anatomical features.

Material & methods

The material studied is deposited in B, KRAM, LPB, UGDA and herb. Flakus. Additional specimens from B, TUR-Vain and TNS were investigated for comparison. Lichen substances were studied by thin-layer chromatography (TLC) using the methods of Culberson & Kristinsson (1970) and Orange et al. (2001). Spot-test reactions with C, K and P were applied to determine the location of secondary metabolites in some specimens.

The genus *Hypotrachyna* in the Neotropics, including some species of *Remototrachyna*, has recently been revised by Sipman et al. (2009), so the descriptions and secondary chemistry of the species are not repeated here. The characteristics of many taxa can also be found in Hale (1975).

Results

The genus *Hypotrachyna*

The diagnostic characters of the genus include the dichotomously or subdichotomously branched lobes with truncate apices, bifusiform conidia, sparsely to richly dichotomously branched rhizines, and an outer exciple consisting of plectenchyma with thin cell walls (Crespo et al. 2010, Divakar et al. 2010). In its current circumscription the genus is paraphyletic (see Blanco et al. 2006, Divakar et al. 2006, Crespo et al. 2010) and needs further study for a better understanding of its relationship to other morphologically similar genera, e.g., *Everniastrum* Hale ex Sipman, *Parmelinopsis* Elix & Hale.

Recently the genus *Remototrachyna* was segregated from *Hypotrachyna* basing mainly on the structure of the cupulate excipulum (plectenchymatous with very thick-walled cells) and genetic differences (Divakar et al. 2010). During our study we investigated several fertile specimens of *Hypotrachyna* from the Neotropics, and in three species, *H. aguirrei*, *H. consimilis* and *H. singularis*, the excipulum appeared typical for *Remototrachyna*; thus those taxa are transferred to *Remototrachyna* (see below).

We also observed that the plectenchyma in the cupulate exciple of *Hypotrachyna* can have thicker cell walls than presented by Divakar et al. (2010), but cell lumina are more or less rounded or ellipsoid, never elongated

and sigmoid in shape as in *Remototrachyna* (for iconography see Divakar et al. 2010).

Hypotrachyna aspera C.H. Ribeiro & Marcelli, in Marcelli & Ribeiro, Mitt. Inst. Allg. Bot. Hamburg 30–32: 133. 2002.

This species has been recently described from Brazil (Marcelli & Ribeiro 2002, Sipman et al. 2009). Here it is reported as new to Bolivia, which represents the first record outside of Brazil.

Specimens examined — **BOLIVIA. Dept. La Paz.** Prov. Franz Tamayo, sendero Keara-Mojos, bajando por la senda de Tokuaqe a Fuertecillo, 2250 m, 14°36′S 68°56′W, 2001, Jimenez 5318 (LPB); **Dept. Tarija.** Prov. Aniceto Arce, Filo de Sidras, 1064 m, 22°14′50″S 64°33′28″W, 2010, Flakus 18431 (LPB, herb. Flakus).

Hypotrachyna dentella (Hale & Kurok.) Hale, Smithsonian Contr. Bot. 25: 33. 1975.

In the Neotropics this species is widespread, but rare; it is also known from the southeastern United States (Hale 1975, Sipman et al. 2009 and literature cited therein). Here the first Bolivian records are presented.

Specimens examined — **BOLIVIA. Dept. Tarija.** Prov. Aniceto Arce, Filo de Sidras, near campamento de guardaparques, 2 hours of Tarija, 1065 m, 22°14′50″S 64°33′28″W, 2010, Flakus 18529, 18615 (KRAM, LPB, UGDA, herb. Flakus); Serranía de Propiedad Arnold, 1309 m, 22°13′19″S 64°33′41″W, 2010, Flakus 18757 (KRAM, LPB).

Hypotrachyna halei Sipman, Elix & T.H. Nash, Fl. Neotrop. Monogr. 104: 76. 2009.

Sipman et al. (2009) reported *H. halei* from Costa Rica, Venezuela, Colombia, and Ecuador; we now report it from Bolivia. The new localities below represent the southernmost known for this species.

Specimens examined — **BOLIVIA. Dept. Cochabamba.** Prov. Chapare, Corani, 3261 m, 17°13′36″S 65°53′25″W, 2009, Flakus 12921 (KRAM, LPB); **Dept. La Paz.** Prov. Franz Tamayo, Piñalito, cerro 29 km en linea recta al Este de Apolo, por el camino a San Jose, 2000-2490 m, 14°29′42″S 68°15′26″W, 2002, Fuentes 5119 (LPB); Prov. Nor Yungas, 4 km del camino principal de Chuspipata hacia Coroico, 2750 m, 16°23′S 67°48′W, 1997, Bach et al. 542 (LPB); PN y ANMI Cotapata, 30 minutes of Unduavi by Sillu Tincara pre-Columbian route, 3437 m, 16°17′38″S 67°53′33″W, 2009, Flakus 16331 & Rodriguez (KRAM, LPB); 5 hours of Unduavi by Sillu Tincara pre-Columbian route, 3429 m, 16°16′33″S 67°52′60″W, 2010, Flakus 16897, 16967, 16972, 16997 & Rodriguez (KRAM, LPB, UGDA, herb. Flakus).

Hypotrachyna lividescens (Kurok.) Hale, Phytologia 28: 341. 1974.

In the Neotropics this species has been reported from Colombia, Guatemala, and Mexico (Sipman et al. 2009), and we now report it from Bolivia. The species is also known from Africa, Australia, and Europe (see Sipman et al. 2009, and literature cited therein). The following record is the southernmost in the Neotropics.

Specimens examined — **BOLIVIA. Dept. Tarija.** Prov. Aniceto Arce, Papachacra, Tucumano-Boliviano montane forest, 2195 m, 21°41′36″S 64°29′33″W, 2010, Flakus 19847, 19848 & Quisbert (KRAM, LPB, herb. Flakus).

Hypotrachyna neoscytodes Elix, T.H. Nash & Sipman, in Sipman et al., Fl. Neotrop. Monogr. 104: 106. 2009.

This species is new to Bolivia and previously known only from the type locality in Colombia (Sipman et al. 2009).

Specimen examined — **BOLIVIA. Dept. La Paz.** Prov. Nor Yungas, Carretera Cotapata-Santa Barbara, Paramo yungueño, 3573 m, 16°19′23″S 67°56′36.8″W, 2007, Rodriguez 453 (LPB).

Hypotrachyna partita Hale, Smithsonian Contr. Bot. 25: 52. 1975.

This species was previously known from Colombia, Costa Rica, Mexico, Panama, Venezuela, and Ecuador, usually from subpáramo dwarf forests (Sipman et al. 2009). The following Bolivian records extend its distributional range southward.

Specimens examined — **BOLIVIA. Dept. La Paz.** Prov. Camacho, Pacoamba cerca Wila Kala, 4286 m, 15°24′40″S 69°04′24″W, 2010, Flakus 17696/3, 17699, 17755 & Rodriguez (KRAM, LPB, UGDA, herb. Flakus); Prov. Nor Yungas, PN y ANMI Cotapata, 5 hours of Unduavi by Sillu Tincara pre-Columbian route, 3429 m, 16°16′33″S 67°52′60″W, 2010, Flakus 16863, 16882 & Rodriguez (KRAM, LPB).

Hypotrachyna primitiva Hale & López-Fig., Bryologist 81: 592. 1979 ["1978"].

H. primitiva was previously known from Colombia and Venezuela (Hale & López-Figueiras 1979, Sipman et al. 2009). The Bolivian localities presented here are the southernmost known and the first records for the Southern Hemisphere.

Specimens examined — **BOLIVIA. Dept. La Paz.** Prov. Franz Tamayo, laguna Tolca Cocha, al NE de Keara Nuevo, 3900 m, 14°41′13″S 69°05′18″W, 2006, Fuentes, Mendoza, Lopez, Madariaga 9927 (LPB, herb. Flakus); Prov. Nor Yungas, Cotapata, 3050 m, 16°17′S 67°51′W, 1998, Franken, Meneses, Villavicencio 6 (LPB).

Hypotrachyna protocetrarica Elix, T.H. Nash & Sipman, in Sipman et al., Fl. Neotrop. Monogr. 104: 127. 2009.

This species, previously known from Ecuador and Peru (Sipman et al. 2009), is reported as new to Bolivia.

Specimens examined — **BOLIVIA. Dept. La Paz.** Prov. Franz Tamayo, laguna Tolca Cocha, al NE de Keara Nuevo, 3900 m, 14°41′13″S 69°05′18″W, 2006, Fuentes, Mendoza, Lopez, Madariaga 9909 (LPB, herb. Flakus); Prov. Murillo, Valle de Zongo, laguna Viscachani, 3840 m, 1989, Coello 6 (LPB); ibid., 1988, Arrazola 29 (LPB).

Hypotrachyna pseudosinuosa (Asahina) Hale, Smithsonian Contr. Bot. 25: 58. 1975.

The widely distributed species in tropical and subtropical regions of the world (Hale 1975, Sipman et al. 2009, and literature cited therein) is recorded for the first time to Bolivia.

Specimens examined — **BOLIVIA. Dept. Cochabamba.** Prov. Chapare, near Incachaca village, 2294 m, 17°14′13″S 65°49′02″W, 2006, Flakus 8222, 8256 (KRAM,

LPB, herb. Flakus); **Dept. La Paz.** Prov. Iturralde, forest above Tumupasa village, 14°08′51″S 67°53′34″W, 350 m, 2008, Kukwa 7023a (UGDA, LPB).

Hypotrachyna subformosana Hale ex Elix, T.H. Nash & Sipman, in Sipman et al., Fl. Neotrop. Monogr. 104: 153. 2009.

This species, widespread but rare in the Neotropics, is reported here as new to Bolivia. It is also known from Fiji (Hale 1975, Sipman et al. 2009).

Specimen examined — **BOLIVIA. Dept. La Paz.** Prov. Nor Yungas, Coroico village, 1550 m, 16°11′10″S 67°43′16″W, 2010, Flakus 16414, 16449 & Rodriguez (KRAM, LPB, herb. Flakus).

The genus *Remototrachyna*

This genus is morphologically very similar to *Hypotrachyna* but differs in having a scleroplectenchymatous cupulate exciple, broad, subirregular lobes with rounded apices, short, mostly dichotomously branched rhizines, a high hymenium, and large ellipsoid ascospores; it is distantly related genetically to *Hypotrachyna* (Divakar et al. 2010). In the course of this study we observed larger variability in exciple structure of *Hypotrachyna* than reported by Divakar et al. (2010). The cells can have thicker walls than previously reported, but in *Remototrachyna* the hyphae are much more thick-walled, with very elongated, thin, and linear to sigmoid cell lumina, which are usually almost vertically orientated.

Basing on anatomical and morphological characters, four additional species were recognized, one new and three transferred from *Hypotrachyna* (see below). Probably more *Hypotrachyna* members should be transferred to *Remototrachyna*, but further investigations of fertile material are needed.

According to Divakar et al. (2010), the ancestral range of *Remototrachyna* was restricted to India. In the light of the new results the genus has a much wider distribution and is more diverse in the Neotropics than previously supposed. Possibly, the ancestral origins of *Remototrachyna* should be reevaluated.

Remototrachyna aguirrei (Sipman, Elix & T.H. Nash) Flakus, Kukwa & Sipman, **comb. nov.**

MycoBank MB 561981

≡ *Hypotrachyna aguirrei* Sipman, Elix & T.H. Nash, Fl. Neotrop. Monogr. 104: 25. 2009.

This species has been recently described from Bolivia, Costa Rica, Colombia, and Peru (Sipman et al. 2009).

Remototrachyna consimilis (Vain.) Flakus, Kukwa & Sipman, **comb. nov.**

MycoBank MB 561982

≡ *Parmelia consimilis* Vain., Acta Soc. Fauna Fl. Fenn. 7(1): 58. 1890.

≡ *Hypotrachyna consimilis* (Vain.) Hale, Smithsonian Contr. Bot. 25: 28. 1975.

Type — [Brasil] Minas Minas Geraës, Caraça, 1885. Vainio, Lich. Brasil. Exs. 1295 (TUR-V 3022 – lectotype, selected by Hale 1975: 28).

This species is known from Mexico, the Caribbean, Venezuela, and Brazil in the Neotropics, and also Taiwan and Papua New Guinea (Hale 1975, Louwhoff & Elix 2002, Sipman et al. 2009 and literature cited therein). Here it is reported as new to Bolivia.

Specimens examined — **BOLIVIA. Dept. Cochabamba.** Prov. Carrasco, Carrasco National Park, near Phaqcha, 20 km of Monte Punku village, 17°27′13″S 65°16′44″W, 2850 m, 2008, Kukwa 6161 (UGDA, LPB); **Dept. La Paz.** Prov. B. Saavedra, Cerro Asunta Pata, 1500 m, 15°05′S 68°29′W, 1997, Bach et al. 204 (LPB).

Additional specimen examined — Vainio, Lich. Brasil. Exs. 1133 (TUR-V 3021, fertile).

Remototrachyna singularis (Hale) Flakus, Kukwa & Sipman, **comb. nov.**

MycoBank MB 561983

≡ *Parmelia singularis* Hale, Phytologia 28: 267. 1974.

≡ *Hypotrachyna singularis* (Hale) Hale, Smithsonian Contr. Bot. 25: 63. 1975.

This rare Neotropical species previously known from Colombia, Ecuador and Peru (Hale 1975, Sipman et al. 2009), is reported as new to Bolivia.

Specimen examined — **BOLIVIA. Dept. Cochabamba.** Prov. Chapare, near Incachaca village, 2317 m, 17°14′11″S 65°49′02″W, 2006, Flakus 8320 (KRAM, LPB, herb. Flakus).

Remototrachyna sipmaniana Kukwa & Flakus, **sp. nov.** PLATE 1

MycoBank MB 561927

Remototrachynae *speciebus medulla acidum protocetraricum et isidiis cortice acida lecanoricum et gyrophoricum continente differt.*

Type — Bolivia, Dept. Cochabamba, Prov. Carrasco, Carrasco National Park, near Phaqcha, 20 km of Monte Punku village, 17°27′13″S 65°16′44″W, 2850 m, Yungas montane cloud forest, close to the river, on rock, 20 July 2008, M. Kukwa 6166 (UGDA-L 15224 – **holotype**; LPB – **isotype**).

Etymology — The species is named after Harrie J.M. Sipman (Botanisches Museum, Berlin Dahlem), an eminent lichenologist dealing with tropical lichens, and co-author of the monograph of the genus *Hypotrachyna* in the Neotropics.

Morphological Characters — thallus saxicolous, 10–20 cm wide, moderately adnate, not coriaceous, irregularly lobate; lobes subirregular, broad, 5–8(–10) mm wide, usually black-rimmed, with entire, crenate, dissected or irregularly incised margins and subrotund apices, usually distinctly ciliate, sometimes with few short and ciliate laciniae; upper surface gray to whitish-gray, sometimes brownish at the tips, smooth or finely rugulose, shiny, in older parts usually delicately cracked, isidiate, lacking soredia, pustules, dactyls or lobules; isidia laminal (mostly present in older parts of the thallus), rarely marginal, scattered to moderately dense, slender, erect, simple to branched, concolorous with thallus, but apices brown, rarely slightly procumbent, intact, few with very short cilia; cilia abundant, black, similar to rhizines, simple to dichotomously branched; medulla white; lower surface black, shiny,

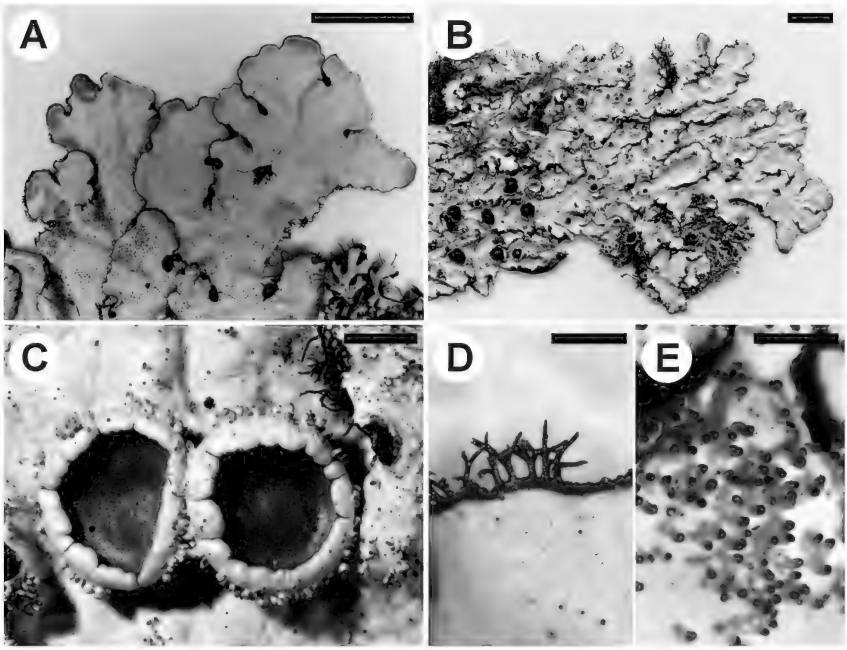

Plate 1. Morphology of *Remototrachyna sipmaniana* (holotype). A: lobes; B: thallus; C: apothecia with isidiate margin; D: branched marginal cilia; E: isidia. Scale bars: A, B = 1 cm; C = 1 mm; D, E = 0.5 mm.

smooth, with a bare brown marginal zone; rhizines moderately dense, but scarce and shorter near the margin, simple (at the margin) or more often up to 3 times dichotomously branched, black to dark brown, with paler apices; apothecia laminal, subpedicellate, 2–5 mm wide; disc concave, light brown to chest-nut brown; thalline margin entire or crenate, concolorous with the thallus, rarely brown near the disc, usually isidiate (isidia shorter and wider than those present on the thallus); cupulate exciple c. 50 µm high, consisting of plectenchyma with thick cell walls; hymenium c. 50 µm high; subhymenium c. 40 µm; asci 8-spored; ascospores hyaline, ellipsoid, 9.5–11 × 5.5–6 µm; pycnidia not seen.

Chemistry — Atranorin (major; thalline and apothecial margin cortex), protocetraric acid (major; medulla of thallus and apothecial margin) and gyrophoric acid (submajor, with minor amounts of lecanoric acid; cortex of isidia). Spot test reaction and fluorescence: upper cortex of thallus and apothecial margin: K+ yellow, C–, P–, UV–; medulla of thallus, isidia and apothecial margin: P+ red-orange, C–; cortex of isidia: P–, C+ pink red.

Distribution and habitat — The new species is known only from two localities in Carrasco National Park, Bolivia. It grows on boulders in Yungas mountain cloud forest.

Additional specimens examined — **BOLIVIA. Dept. Cochabamba.** Prov. Chapare, near Incachaca village, 17°14′09″S 65°48′51″W, 2198 m, Yungas montane cloud forest, on rock, 2006, Flakus 7852 (B, KRAM, LPB, herb. Flakus).

Reference material examined — **JAPAN. Prov. Kii:** Koya. Saxicolous. October 19, 1952. Coll. Y. Asahina 52101 (TNS – isolectotype of *Parmelia koyaensis*).

Comments — *Remototrachyna sipmaniana* is distinguished from all other species of the genus by the abundant, branched cilia. However, the structure of the cupulate exciple and the general morphology agree with the diagnostic characters of *Remototrachyna*.

The species shows considerable variation in the production of isidia. In the holotype they are very abundant on the thallus as well as on the apothecial margin, but in the paratypes isidia are less frequent; in some thalli they are sparse and many apothecia are not isidiate.

Remototrachyna sipmaniana is also distinguished by the production of protocetraric acid in the medulla of the thallus, isidia and apothecial margin whereas gyrophoric acid is restricted to the cortex of the isidia. Three other isidiate species of *Remototrachyna*, *R. incognita* (Kurok.) Divakar & A. Crespo, *R. consimilis* and *R. koyaensis* (Asahina) Divakar & A. Crespo are chemically similar, but differ in other characters. *Remototrachyna incognita* contains gyrophoric acid, but together with protolichesterinic acid (Divakar & Upreti 2005), whereas *R. sipmaniana* produces protocetraric and gyrophoric acids; the former occurs in Asia (Divakar & Upreti 2005), while the latter occurs in the Neotropics.

Both *R. consimilis* and *R. koyaensis* contain protocetraric acid (e.g., Hale 1975, Divakar & Upreti 2005, Sipman et al. 2009), and although gyrophoric acid has been detected in some specimens in minor or trace amounts, the reaction with C has been always reported as negative (Louwhoff & Elix 2002, Sipman et al. 2009). *Remototrachyna consimilis* has very similar ascospore dimensions to *R. sipmaniana* (see Sipman et al. 2009), but it differs by having much narrower lobes, (0.5–)1–2(–3) mm wide, and partly yellow-pigmented lower medulla (Sipman et al. 2009). *Remototrachyna koyaensis* can be readily separated by the production of caperatic acid (absent in *R. sipmaniana*) and the ascospore size (9.5–11 × 5.5–6 µm in *R. sipmaniana*; versus more than 14 µm long and 7 µm wide in *R. koyaensis*) (Louwhoff & Elix 2002, Divakar & Upreti 2005, Sipman et al. 2009, sub *Hypotrachyna*). Additionally, they differ in distribution: *R. koyaensis* occurs in Asia, Papua New Guinea and in Mexico (Louwhoff & Elix 2002, Sipman et al. 2009), whereas *R. sipmaniana* occurs in Bolivia. We examined the types of *Parmelia consimilis* and *P. koyaensis* and did not detect

gyrophoric acid by TLC nor did the isidia react with C. Possibly gyrophoric acid is a relatively rare accessory substance in *R. consimilis* and *R. koyaensis*, but the chemical variability needs to be studied in more collections.

In the Neotropics *R. sipmaniana* could be also confused with two other isidiate species, *R. costaricensis* (Nyl.) Divakar & A. Crespo and *R. rhabdiformis* (Kurok.) Divakar & A. Crespo. However, both have alternative chemistry, the former produces fatty acids and the latter norstictic, salazinic and consalazinic acids (Sipman et al. 2009).

The new species could also be mistaken for some chemically similar isidiate species of *Hypotrachyna*, but they do not contain major quantities of both protocetraric and gyrophoric acids in the same thallus, nor are they distributed as in *R. sipmaniana*.

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Mycena moconensis, a new species in section *Polyadelphia* from Argentina

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Abstract — A new agaric species, *Mycena moconensis*, is described from Paranaense Rain Forest, Argentina. This species is characterized by small bicoloured basidiomata with yellow to orange yellow or golden pilei and magenta to purple stipes, pip-shaped spores, and spinulose cheilocystidia and pellicular hyphae.

Key words — *Agaricomycetes, Agaricales, Mycenaceae*, new taxa, taxonomy

Introduction

Mycena (Pers.) Roussel comprises approximately 500 species, widely distributed in the world (Kirk et al. 2008). It is a polyphyletic genus where comprehensive molecular analyses are needed to clarify the infrageneric classification and species circumscriptions.

South American species of *Mycena* have been studied by Spegazzini (1887, 1898), Rick (1938), Dennis (1961), Singer (1969, 1973, 1989), Valenzuela & Moreno (1995), Raithelhuber (1984a,b, 1985a–f, 1996a,b, 2004) and Maas Geesteranus & de Meijer (1997, 1998). Raithelhuber (1985f, 1996a) published monographic studies that included identification keys to *Mycena* species in the region. Maas Geesteranus & de Meijer (1997) also provided a key to species described or mentioned previously by Singer.

There are 114 *Mycena* species so far recorded in Argentina. Most have been found in the Andino-Patagonic forest and the Yungas region of northwestern Argentina, with only six species known from the Paranaense forest (Spegazzini 1926, Wright et al. 2008, Lechner et al. 2006, Wright & Wright 2005, Niveiro et al. 2010).

According to Maas Geesteranus, "many more species of *Mycena* await to be discovered in Paraná State (Brazil) but, however desirable, the search cannot for various reasons be continued" (Maas Geesteranus & de Meijer 1998).

During a survey of the *Agaricomycetes* of northern Argentina, we recently collected some specimens that do not match any other known species of *Mycena*. We propose herein *M. moconensis,* a new species in section *Polyadelphia*.

Material & methods

Macroscopic description is based in fresh material, according to Largent (1986) and Lodge et al. (2004). Microscopic features are described from material mounted in KOH 5%, phloxine (1%), and Melzer's reagent. The following notations are used: L = number of lamellae reaching the stipe; x = arithmetic mean of the spore length and width; Q = quotient of length and width indicated as a range of variation; Qm = mean of Q values; n = number of spores measured. Herbarium specimens are deposited in CTES, BAFC, and LIL. Herbarium abbreviations follow Holmgren et al. (1990).

Results

Mycena moconensis Niveiro, Albertó & Desjardin, **sp. nov.** FIGS 1–8

MycoBank MB519458

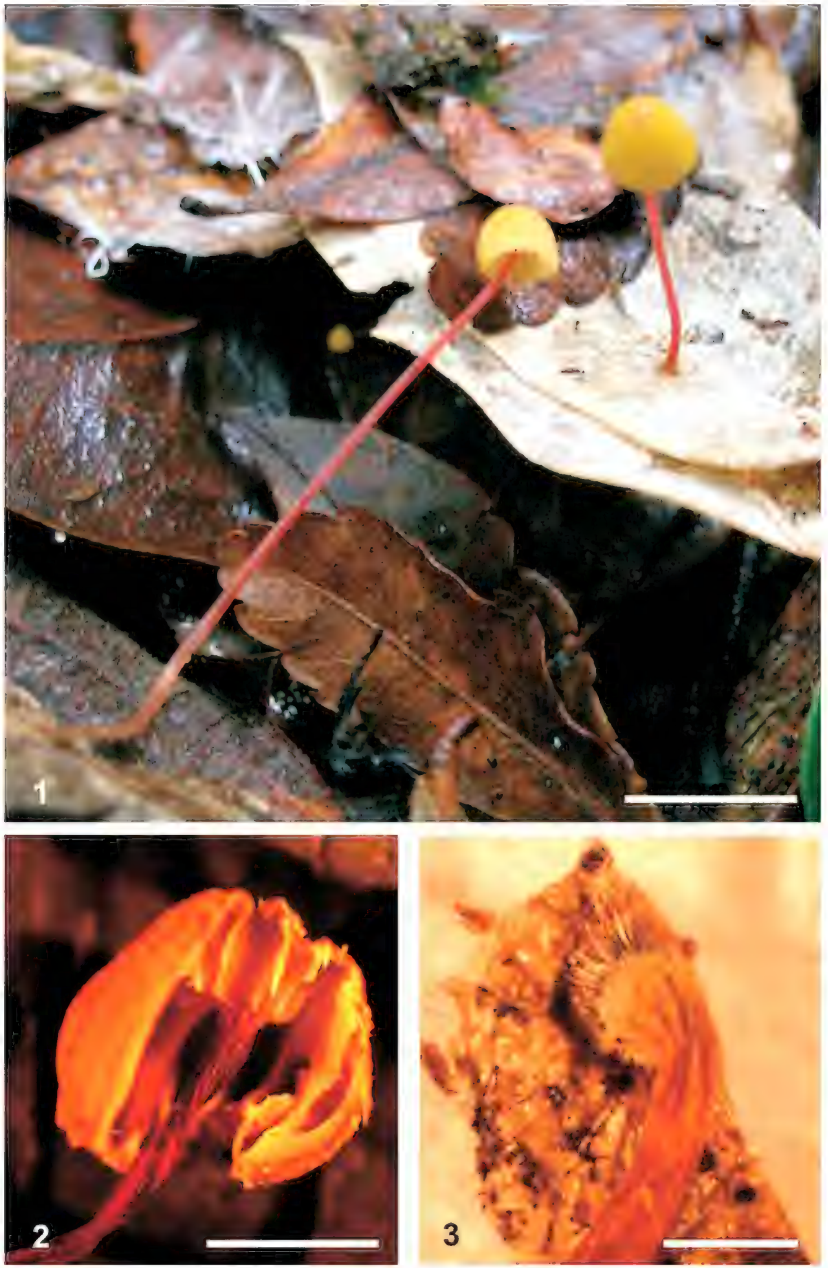
Differing from other species of *Mycena* sect. *Polyadelphya* by its yellow to golden pileus and magenta to purple stipe.

TYPE: Argentina, Misiones, Parque Provincial Moconá, Sendero de la Gruta, (27°09′13.2″S 53°54′04.7″W), 17.V.2008, leg. N. Niveiro et al. 767, (**Holotype**, CTES 0591002).

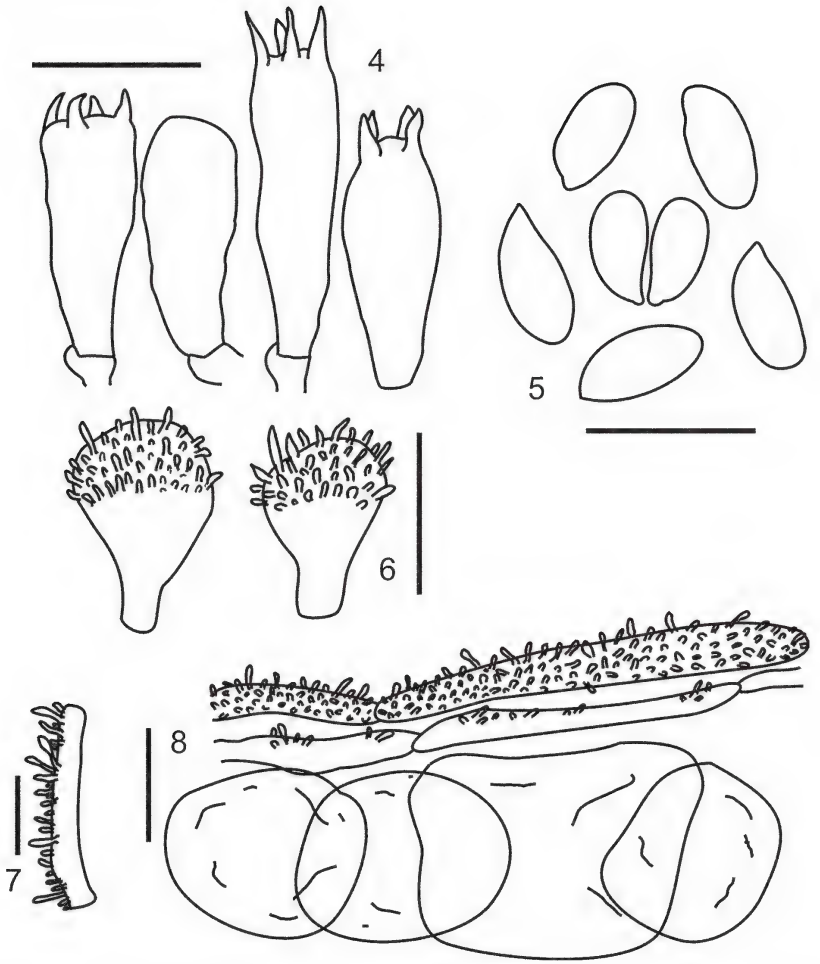
ETYMOLOGY: From Moconá Provincial Park.

BASIDIOMATA scattered to gregarious. PILEUS 1–8 mm in diameter, broadly parabolical to hemispherical or campanulate, without umbo; margin entire, decurved, translucent striate to sulcate; surface smooth, slightly pruinose to silky, dry; yellow to orange yellow or golden. CONTEXT thin. ODOR and TASTE absent. LAMELLAE adnate to slightly decurrent by a tooth, subdistant (L = 11–13) without lamellulae, up to 0.5 mm wide, white; entire edge, concolorous. STIPE 30–60(–90) × 0.5–1.5 mm, central, cylindrical, equal, hollow, smooth, glabrous, apex magenta to dark magenta, purple or Indian red, paler on the base; attached to the substratum by a mycelial pad of brown radiating hyphae. SPORE PRINT white.

BASIDIOSPORES 5.7–8.6 × 3–4.5 µm, [x = 7.5 × 4 µm, Q = 1.59–2.27; Q_m = 1.87, n = 38), oblong to pip-shaped, smooth, hyaline, amyloid, thin-walled, without germ pore. BASIDIA 20–23 × 6–7 µm, clavate, 4-sterigmate. PLEUROCYSTIDIA absent. CHEILOCYSTIDIA 10–16 × 7–10 µm, hyaline, broadly clavate to subglobose, densely spinulose, with simple cylindrical excrescences 2–2.6 µm long. HYMENOPHORAL TRAMA regular; hyphae 2.5–11 µm diam, dextrinoid, non-gelatinous. PILEIPELLIS a thin cutis of repent, radially aligned hyphae; hyphae 2.5–5.5 µm diam., densely covered with short cylindrical



Figs 1–3. *Mycena moconensis*: 1. General aspect; 2. Detail of pileus; 3. Stipe with mycelial filaments radiating from the base. Scale bars: 1 = 20 mm; 2 = 2 mm; 3 = 1 mm.



Figs 4–8. *Mycena moconensis*: 4. Basidia; 5. Basidiospores; 6. Cheilocystidia; 7. Hyphae of the stipitipellis; 8. Pileipellis and hypoderm. Scale bar = 10 µm.

spinulae, non-gelatinous. Hypodermium pseudoparenchymatous, with cells 18–37 µm diam., dextrinoid. stipitipellis hyphae 3.5–5.5 µm diam., densely covered with short cylindrical spinulae. Caulocystidia absent or rare and scattered. Clamp connections present.

Habitat: Gregarious in small clusters, on leafy litter.

Additional specimens examined — **ARGENTINA, Misiones**, Parque Provincial Moconá, Sendero de la Gruta, (27°09′17.1″S 53°54′02.6″W), 25/V/2009, Niveiro & Michlig 1276 (BAFC); 25/V/2009, Niveiro & Michlig 1296 (LIL).

Discussion

This beautiful new species is characterized by small bicolored basidiomata with yellow to orange yellow or golden pilei and magenta to purple stipes.

The following combination of features refer this species to section *Polyadelphia* (Maas Geesteranus 1986, Maas Geesteranus & de Meijer 1997): small basidiomata with few lamellae, thin stipes with radiating basal mycelium, cheilocystidia densely covered with simple excrescences, pip- shaped basidiospores, and a non-gelatinous pileipellis composed of hyphae densely ornamented with small spinulae.

Mycena moconensis could be confused with "*M. coprinoides*" P. Karst. sensu Raithelh., a species previously recorded in Argentina (Raithelhuber 2004). The latter species differs, however, in forming a pale yellow to pale ochraceous pileus with a weakly striate margin, crowded and adnexed-adnate lamellae, a pale yellow stipe with a darker base, and fusiform cheilocystidia. It should be noted that Maas Geesteranus (1981) considers *Mycena coprinoides* P. Karst. as representing a species of *Coprinus*, based on his examination of the holotype specimen. The taxon reported by Raithelhuber (2004) as "*M. coprinoides*" may represent an undescribed species.

The presence of a citron yellow to barium yellow pileus, sulcate-striate margin, adnate-decurrent, distant lamellae, and pyriform to clavate cheilocystidia with excrescences also diagnose *Mycena citricolor* (Berk. & M.A. Curtis) Sacc., a pathogen of coffee known from the New World tropics. *Mycena citricolor* differs in forming a yellow stipe, slightly smaller basidiospores (4.2–6 × 2.3–3.5 µm), and pileipellis hyphae that are embedded in a gelatinous matrix (Pegler 1983). It also differs in forming bioluminescent mycelium on which are produced stilboid asexual propagules with the anamorphic *Decapitatus flavidus* (Cooke) Redhead & Seifert.

The similar *M. melinocephala* Singer, described from Argentina on *Alnus* wood, differs in forming a yellowish honey-coloured pileus and stipe and larger, subglobose to globose basidiospores (8–10.5 × 7.5–9.5 µm) (Singer 1973). *Mycena ixoxantha* Singer, a species described from Colombia with citrine yellow pileus and amber coloured stipe, differs in forming gelatinous pileipellis tissue (Singer 1973).

Two species from the northern hemisphere, *Mycena albiceps* (Peck) Gilliam and *M. capillaris* (Schumach.) P. Kumm., share similar micro-morphological features with *M. moconensis* but differ in forming white to pale greyish white pilei and dark greyish brown to black stipes (Maas Geesteranus 1986).

Maas Geesteranus & de Meijer (1997, 1998) described two species of *Mycena* sect. *Polyadelphia* — *M. elongata* Maas Geest. & de Meijer and *M. tuberifera* Maas Geest. & de Meijer — from Paraná State (Brazil) near the location where *M. moconensis* was found. They are characterized by very dark brown pilei and

a pale yellow (*M. elongata*) or white (*M. tuberifera*) stipe. The authors regarded both species as unusual for section *Polyadelphia* by differing from northern hemisphere representatives in forming smooth stipitipellis hyphae and simple, non-spinulose caulocystidia, characters not observed in *M. moconensis*.

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A new species of *Fulvifomes* (*Hymenochaetaceae*) from Cambodia

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Abstract — *Fulvifomes cambodiensis* sp. nov. is described and illustrated from Preah Vihear, Cambodia. It produces perennial, pileate and solitary basidiomes with a concentrically sulcate zonate pileal surface, homogeneous context, hymenial setae, and yellowish acyanophilous basidiospores. The new species macroscopically resembles *F. rimosus,* which differs in lacking hymenial setae and having cyanophilous generative hyphae and slightly smaller basidiospores. The macroscopically similar *Fomitiporia hartigii* is distinguished by larger hyaline basidiospores and the absence of setae. *Fulvifomes johnsonianus* has abundant hymenial setae and quite similar hyphal structure to *F. cambodiensis,* but its much smaller basidiospores and effused-reflexed to pileate basidiomes distinguish it from the new species with pileate and ungulate basidiomes with cracked pileal surface.

Key words — *Hymenochaetales,* polypore, taxonomy

Introduction

After Murrill (1914) segregated *Fulvifomes* Murrill (*Hymenochaetaceae* Donk), typified by *F. robiniae* (Murrill) Murrill, from *Phellinus* Quél., many mycologists did not accept his genus (Ryvarden & Johansen 1980, Gilbertson & Ryvarden 1987, Ryvarden & Gilbertson 1994, Núñez & Ryvarden 2000), although it was treated as a subgenus of *Phellinus* by Dai (1999). Recent molecular phylogenetic analyses clearly support it as an independent genus closely related to *Aurificaria* D.A. Reid and *Phylloporia* Murrill (Wagner & Fischer 2002, Larsson et al. 2006). Morphologically, while all three genera produce colored basidiospores, the dimitic hyphal system of *Fulvifomes* distinguishes it from the two monomitic genera (Dai 2010). Except for the dimitic hyphal system and colored basidiospores, *Fulvifomes* species have few distinctive morphological characters (Dai 2010).

During a study on wood-decaying fungi in Cambodia, an unknown *Fulvifomes* species was discovered, which is here described and illustrated as *Fulvifomes cambodiensis*. Morphological differences between *F. cambodiensis* and other similar species according to Chinese materials are also noted.

Materials & methods

The studied specimen was deposited at the herbaria of Guangdong Institute of Microbiology (GDGM) and Institute of Applied Ecology, Chinese Academy of Sciences (IFP). The microscopic procedure follows Dai (2010). Sections were studied at magnification up to ×1000 using a Nikon Eclipse E 80i microscope and phase contrast illumination. The variation in the size of the spores was presented by excluding 5% of measurements from each end of the range, which were given in parentheses. The abbreviations are used as follows: IKI = Melzer's reagent, IKI– = negative in Melzer's reagent, KOH = 5% potassium hydroxide, CB = Cotton Blue, CB– = acyanophilous, L = mean spore length (arithmetic average of all spores), W = mean spore width (arithmetic average of all spores), Q = variation in the L/W ratios within the specimen studied, and n = number of spores, hyphae or setae measured from given number of specimens. Line drawings were made with the aid of a light tube. Special color terms follow Anonymous (1969) and Petersen (1996).

Taxonomy

Fulvifomes cambodiensis L.W. Zhou & W.M. Zhang, **sp. nov.** FIGS 1–2

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Differs from *Fulvifomes rimosus* in the presence of hymenial setae, slightly larger basidiospores, and acyanophilous generative hyphae.

TYPE: Cambodia, Preah Vihear, on angiosperm wood, 12.V.2006, GDGM 20949 (**holotype** in GDGM, **isotype** in IFP).

ETYMOLOGY: *cambodiensis* (Lat.): refers to the country of Cambodia.

BASIDIOMES: Perennial, pileate, ungulate, in section triquetrous, usually solitary, woody hard and without odour or taste when dry. Pileus projecting up to 6 cm, 15 cm wide and 8 cm thick at base; pileal surface deep reddish brown to dark clay-buff, glabrous, concentrically sulcate with wide zones, glabrous with age, cracked to slightly rimose with age, margin obtuse. Pore surface yellowish brown, sterile margin yellowish brown, up to 10 mm; pores round to angular, 5–6 per mm; dissepiments thin, entire. Context cinnamon, woody hard, up to 13 mm thick. Tubes cinnamon, woody hard, tube layers distinct, annual layer up to 3 mm long.

HYPHAL STRUCTURE: Dimitic; generative hyphae without clamp connections; skeletal hyphae IKI–, CB–; tissues darkening but otherwise unchanged in KOH.

CONTEXT: Generative hyphae scanty, hyaline, thin-walled, occasionally branched, 2–3 μm in diam; skeletal hyphae dominant, yellowish brown, thick-

FIGURE 1. *Fulvifomes cambodiensis* basidiocarp (holotype).

walled, flexuous, unbranched, without a simple septum, tightly interwoven, 3–5 µm in diam (n = 10/1).

TUBES: Generative hyphae frequent, hyaline, thin-walled, occasionally branched, frequently septate, sometimes diminishing at the end or middle, 1.7–2.5 µm in diam; skeletal hyphae dominant, yellowish brown, thick-walled with a distinct lumen to subsolid, flexuous, unbranched, interwoven, sometimes agglutinated, 2–3.5 µm in diam (n = 10/1). Hymenial setae frequent, dark brown, subulate to ventricose, thick-walled, 12.7–30.4 × 6.5–17 µm (n = 10/1). Hymenium collapsed, cystidia, cystidioles, basidia and basidioles not seen.

SPORES: Basidiospores broadly ellipsoid, yellowish, thick-walled, smooth, CB–, IKI–, (5.5–)5.8–6.2(–6.3) × (4.5–)4.6–5.2 µm, L = 5.98 µm, W = 4.95 µm, Q = 1.21 (n = 30/1).

REMARKS: *Fulvifomes cambodiensis* is macroscopically characterized by solitary perennial, pileate, woody hard basidiomes with a crust-covered and concentrically sulcate pileal surface and homogeneous context; microscopically it has hyaline thin-walled generative hyphae, yellowish brown thick-walled skeletal hyphae, hymenial setae, and yellowish acyanophilous basidiospores.

Fulvifomes rimosus (Berk.) Fiasson & Niemelä shares with *F. cambodiensis* a perennial pileate ungulate growth habit, concentrically sulcate zonate pileus,

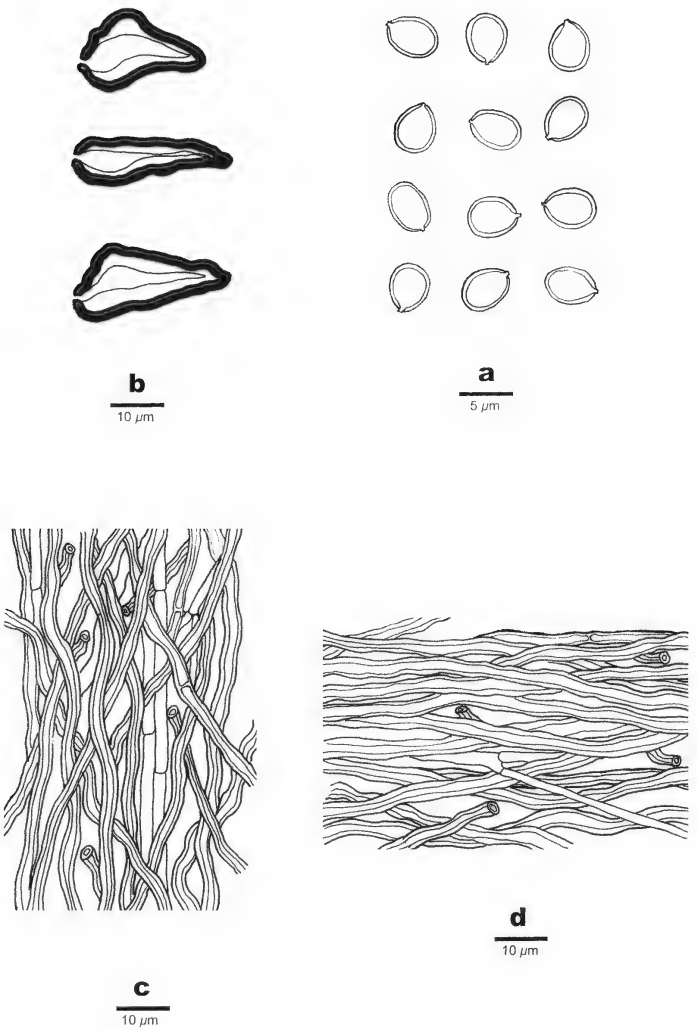

FIGURE 2. *Fulvifomes cambodiensis* (holotype).
a: Spores. b: Setae. c: Hyphae from trama. d: Hyphae from context.

and obtuse pileal margin. However, the basidiomes of *F. rimosus* are darker. Microscopically, *F. rimosus* lacks hymenial setae and has slightly smaller basidiospores (L = 5.54 μm, W = 4.54 μm) and cyanophilous generative hyphae (Dai 2010).

The macroscopically similar *Fomitiporia hartigii* (Allesch. & Schnabl) Fiasson & Niemelä shares with *F. cambodiensis* the perennial and ungulate basidiomes with the concentrically sulcate cracked pileal surface, obtuse pileus margin, and almost identical pore sizes (Dai 2010), making the two species very hard to differentiate in the field. But *F. hartigii* is distinguished by its wider hyphae, lack of setae, and larger hyaline basidiospores that are dextrinoid and strongly cyanophilous (Dai 2010).

Fulvifomes johnsonianus (Murrill) Y.C. Dai has quite similar hyphal structure to *F. cambodiensis,* and the two species also share abundant hymenial setae and ellipsoid basidiospores (Q = 1.23, Dai 2010). However, *F. johnsonianus* has much smaller basidiospores (2.8–3.2 × 2.2–2.6 μm; Dai 2010). Macroscopically, *F. johnsonianus* has effused-reflexed to pileate basidiomes, which are easily differentiated from the pileate and ungulate basidiomes with cracked pileal surface of *F. cambodiensis* (Dai 2010).

Acknowledgements

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Fungal pathogens of 'cat's claws' from Brazil for biocontrol of *Macfadyena unguis-cati*

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Abstract—Recent searches in Brazil for plant pathogens with potential for use as classical biocontrol agents of *Macfadyena unguis-cati* yielded five fungal species, two previously undescribed. These are described herein and preliminary evaluations based on field and laboratory observations of the diseases with which they are associated are given. These are: *Guignardia mangiferae* (leaf spots), *Meliola herteri* (black mildew), *Passalora macfadyenae* sp. nov. (leaf spots), *Pseudocercospora unguis-cati* (leaf spots) and *Prospodium macfadyenae* sp. nov. (rust). The two newly described species, *Pa. macfadyenae* and *Pr. macfadyenae*, are regarded as having the greatest potential for use in classical biological control.

Key words —cat's claws, new species, plant pathology, taxonomy

Introduction

Invasion of natural ecosystems by exotic species represents one of the biggest threats to biodiversity, as now acknowledged by scientists and governments throughout the world (IUCN 2000). Once an alien organism has invaded, the sole long-term sustainable management alternative available for mitigating it through human intervention is the introduction of natural enemies collected in the pest's centre of origin in a process known as classical biological control. *Macfadyena unguis-cati* (L.) A.H. Gentry (*Bignoniaceae*) — common name cat's claws (in Brazil: unha-de-gato, unha-de-morcego) — is a woody vine that has a broad distribution in its native range occurring from southern Brazil and Argentina to Mexico (Everett 1980). *Macfadyena unguis-cati* takes its popular names from the tendrils, which have acute divided ends that appear and function like claws, allowing the plant to firmly attach to trees or other supports on which it grows (Plate 1). At maturity it produces abundant showy yellow flowers making it a useful ornamental plant for cultivation on fences

and garden walls (Lorenzi & Souza 1995). Because of its value as an ornamental it was introduced to regions outside its native range, where it escaped from gardens to become a noxious invader of native forests, particularly in the USA, Australia, China, and South Africa (Henderson 1995, FLEPPC 2005, Williams et al. 2008, Osunkoya et al. 2009, Dhileepan et al. 2010). Its vigorous vine habit allows it to ascend tall trunks reaching the treetops (Plate 2) branching and spreading over top of the forest canopy. The combination of the biomass weight and shading of underlying vegetation can be very harmful and even cause the death of the largest plants in infested areas and suppress the development of smaller plants and seed germination of native plants (Neser 1996, Huang et al. 2009, King & Dhileepan 2009). In Florida, *M. unguis-cati* is listed as a Category 1 weed, a rank reserved for species capable of altering plant communities by substituting and even permanently changing the natural structure of an ecosystem and its ecological functions (FLEPPC 2005). Between 2003 and 2005 a search was conducted in native situations in Brazil for fungal pathogens attacking *M. unguis-cati* that might have potential as biocontrol agents. This publication presents the results of this preliminary survey with particular reference to fungal taxonomy and the potential to use mycobiota in classical biological control.

Material & methods

Prior to the field survey, herbarium records were completely searched for occurrence and distribution of *M. unguis-cati* in the following Brazilian herbaria: Herbário Fanerogâmico e Criptogâmico do Instituto Agronômico (IAC); Herbário Maria Eneyda P. K. Fidalgo (SP); Instituto de Pesquisas Jardim Botânico do Rio de Janeiro (RB); Herbário do Museu Nacional do Rio de Janeiro (R); Universidade Federal de Viçosa (VIC); Museu Botânico Municipal (MBM); and Herbário José Badini (OUPR). Using this information, we prepared an itinerary for surveying the southern and southeastern Brazilian states Minas Gerais, Rio de Janeiro, Espírito Santo, São Paulo, Paraná, Santa Catarina, and Rio Grande do Sul. Surveys were conducted between January 2003 and December 2005. Selected sites were visited and carefully scrutinized for the presence of *M. unguis-cati*. For details on the general survey and laboratory methodologies see Barreto & Evans (1994). Freshly collected samples were examined under a stereomicroscope. Slides containing free-hand sections of colonized tissues or fungal structures scraped from the surface of infected tissues were mounted in lactophenol or lactofuchsin. Isolations of non-biotrophic fungi were attempted by directly transferring spores or other fungal structures to plates containing Vegetable Broth Agar (VBA, Pereira et al. 2003), under a dissecting microscope with a sterilized fine point needle. Observations, measurements, photographs and line drawings were prepared using an Olympus BX 50 light microscope fitted with a camera (Olympus Evolt SC 50) and drawing tube.

For scanning electron microscopy the samples were fixed in 2.5% glutaraldehyde + sodium cacodylate buffer (0.1mol L^{-1}, pH 7.2) (1:1) for 1h at room temperature, washed six times in 0.1mol L^{-1} sodium cacodylate buffer (10 m each washing period), post-fixed

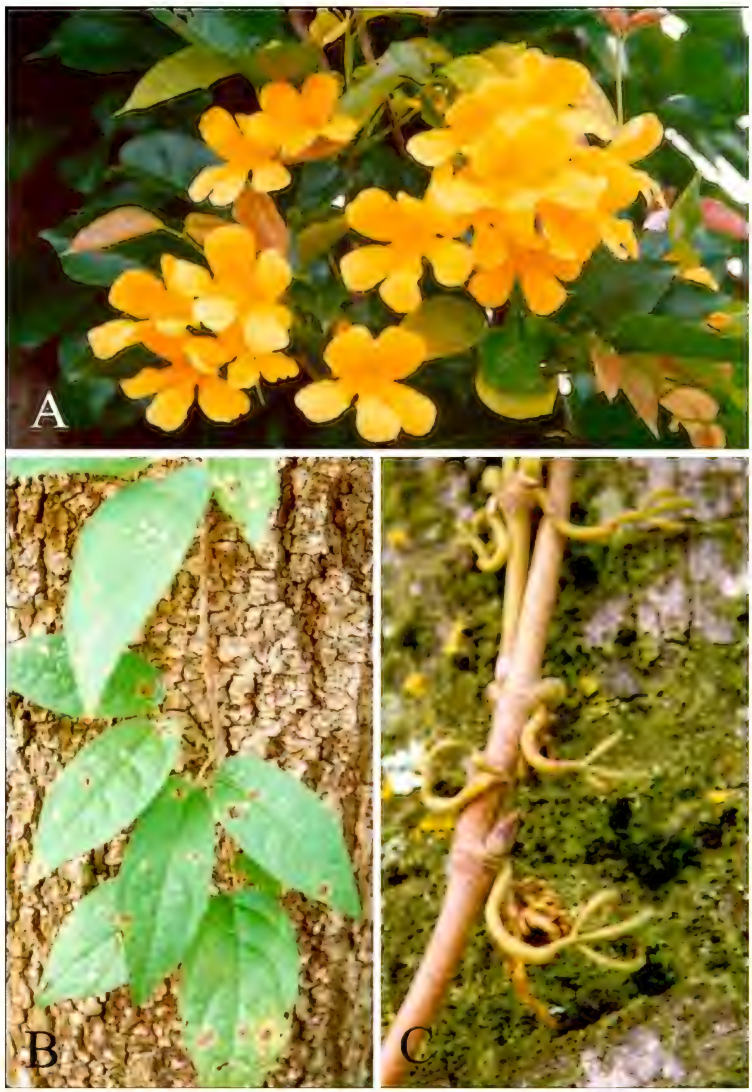

PLATE 1. *Macfadyena unguis-cati*: A. typical large, yellow, trumpet-shaped flowers; B. climbing on tree trunks; C. clawed tendrils which enable plant to climb up against walls, tree trunks, and other supports.

by immersion in 1% O_sO_4 prepared in cacodylate buffer 0.1 mol L^{-1} (1:1), and kept for 4 h at 4-8 °C then washed again six times in buffer, dehydrated by 10-min long successive transfers in an alcohol solution (30, 50, 60, 70, 80, 95, 100%) gradation series at room temperature, dried in a critical point dryer (Baltec model 030) with CO_2 as transition

Plate 2. *Macfadyena unguis-cati* climbing trees in the field in Brazil.

fluid, and coated with 20 nm thick gold in a sputter coater (Balzers® model FDU 010) before examination in a Carl-Zeiss Model LEO VP 1430 electron microscope.

Representative specimens were deposited in the herbarium at the Universidade Federal de Viçosa (Herbarium VIC).

Taxonomy

Numerous samples of diseased *M. unguis-cati* were collected during the survey. Five different fungal pathogens were recognized in association with cat's claws. Two fungal species were isolated in pure culture. The five fungal pathogens were associated with the following diseases: leaf spots (3), black mildew (1), and rust (1). Descriptions of the identified fungi are given below.

Guignardia mangiferae A.J. Roy, Indian Phytopath. 20: 348. 1968. PLATE 3

Lesions on living leaves, minute dark tar-spots corresponding to fungal conidiomata surrounded by faint chlorotic areas, occasionally associated to larger necrotic areas. External mycelium absent. Pseudothecia hypophyllous, immersed, solitary or in groups, subglobose, 136.5–113 µm, wall composed of texture angularis, 17.5–27.5 µm, dark brown to black, smooth. Asci bitunicate, subclavate to cylindrical, 46.5–82 × 11–14 µm, eight-spored. Ascospores biseriate, fusiform-elliptical, 12–14 × 4.5–5 µm, unicellular, guttulate, hyaline, smooth, surrounded by a thick mucilaginous coat.

MATERIAL EXAMINED: **BRAZIL. SÃO PAULO**, on *Macfadyena unguis-cati*, 16 April 2005, OLPereira (VIC 30492); **MINAS GERAIS**, on *Macfadyena unguis-cati*, 25 July 2011, MSilva & OLPereira (VIC 31759).

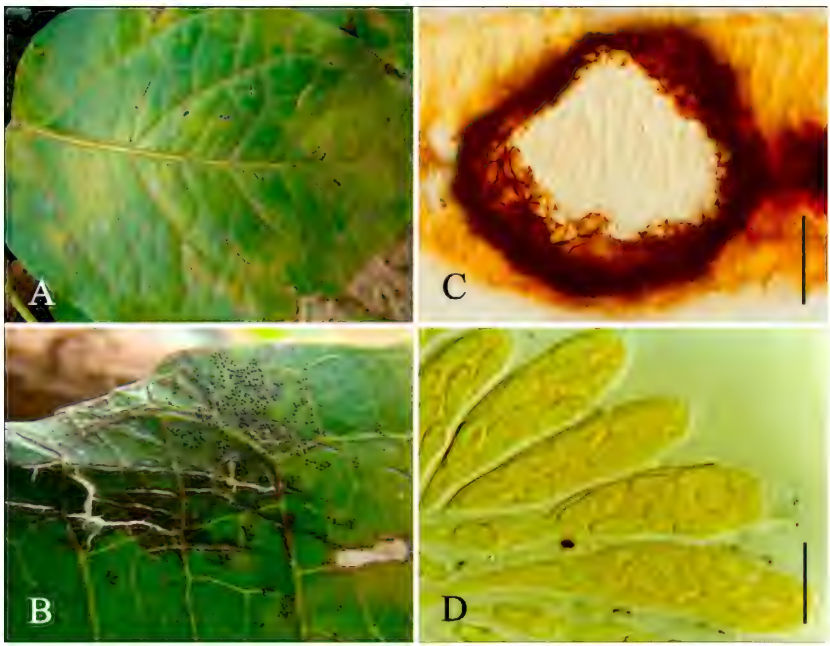

PLATE 3. A–B: *Guignardia mangiferae* on *Macfadyena unguis-cati*. A. Faint chlorotic symptoms on infected leaves; B. circular individual pseudothecia on adaxial leaf surface. C–D: *Guignardia mangiferae* (VIC 30492). C. Section through a pseudothecium showing the asci; D. bitunicate asci containing biseriate, hyaline ascospores. Bars: C = 30 µm; D = 10 µm.

Comments: *Guignardia mangiferae* was observed causing some damage to few isolated leaves on infected *M. unguis-cati* plants (Plate 3A–B) but is regarded here as having no potential for classical biocontrol of *M. unguis-cati*. This is the first record of *G. mangiferae*, a polyphagous opportunistic pathogen of numerous plant families (Rodrigues et al. 2004), on a representative of *Bignoniaceae* (*M. unguis-cati*) worldwide.

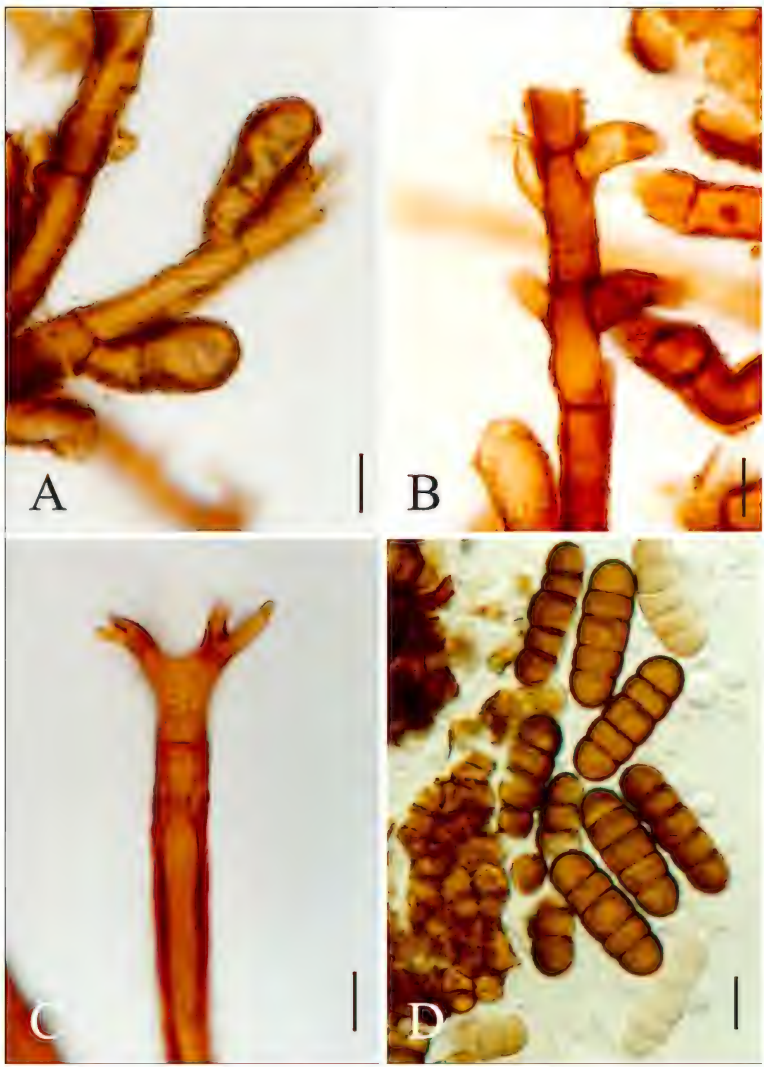

Plate 4. *Meliola herteri* (VIC 30485): A. external hyphae with alternate appressoria; B. opposite conidiogenous cells; C. branching-dentate setae; D. ascospores. Bars: A–C = 10 µm; D = 20 µm.

Meliola herteri Hansf., Sydowia 9: 65. 1955. PLATE 4

Colonies amphigenous, mostly epiphyllous, 4–50 mm diam, sooty, scattered, numerous, confluent, black. External mycelium net-forming, 6–9.5 µm diam hyphae, slightly undulate, alternately branching at acute angles, dark brown, septate, producing phialides (mucronate hyphopodia) and appressoria (capitate hyphopodia), smooth, brown; appressoria alternate apical, stalk-cells cylindrical 6–16 × 5.5–9.5 µm, head-cells oval or elliptical, 14–19 × 9.5–14 µm dark brown; phialides intermixed with appressoria, ampulliform to cylindrical, 14–25.5 × 5.5–9 opposite or alternate, scarce. Perithecia superficial, aggregate, globose, 112–155 µm diam. Mycelial setae scattered, straight to slightly curved, 155–253 × 6–9.5 µm, the apex usually 2–4 furcate and secondarily 2–3 branched, brown. Asci evanescent at maturity. Ascospores cylindric to oblong, 44–50.5 × 13–19 µm, constricted at the septa, apex and base rounded, 4-septate, brown, smooth.

MATERIAL EXAMINED: **BRAZIL. RIO GRANDE DO SUL**, on *Macfadyena unguis-cati*, 6 August 2004, OLP (Pereira) (VIC 30485).

COMMENTS: Three *Meliola* species are known to occur on *M. unguis-cati*: *Meliola thaxteri* Hansf., *M. bidentata* Cooke, and *M. herteri* (Hansford 1961). The morphology of the specimen collected on *M. unguis-cati* during the survey clearly placed this fungus within *M. herteri* as described by Hansford (1961). *Meliola herteri* has been previously recorded on *M. unguis-cati* in Paraguay, *Amphilophium vauthieri* DC. in Argentina, and *Parabignonia maximilianii* (Mart ex DC.) Bureau in Brazil (Hansford 1961). This is the first report of *M. herteri* on *M. unguis-cati* in Brazil. *Meliola herteri* is, as are the *Meliolaceae* in general, a weak obligate biotroph and the black mildew diseases the family causes do not significantly affect their hosts (Hansford 1961). This fungal group therefore has little relevance for use in biological control.

Passalora macfadyenae Meir. Silva, O.L. Pereira & R.W. Barreto, **sp. nov.** PLATE 5

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Morphologically similar to *Passalora adenocalymmatis* and *Passalora pyrostegiae* but differs in shorter (31.5–114 µm) conidia, longer (16-114 µm) conidiophores, and a well-developed stroma.

TYPE: Brazil, Minas Gerais, on *Macfadyena unguis-cati*, 18 May 2004, OLP (Pereira) (VIC 30480, **holotype**).

ETYMOLOGY: from the host genus name.

Lesions on living leaves, similar to those cause by *P. unguis-cati* but somewhat darker centrally, 4–55 mm in diam., coalescing to cover large areas of leaf surface and leading to leaf blight. Internal mycelium indistinct. External mycelium absent. Stromata erumpent, 63–142 × 35–95 µm, dark brown. Conidiophores hypophyllous arising from stromata, in dense fascicles, cylindrical, straight to

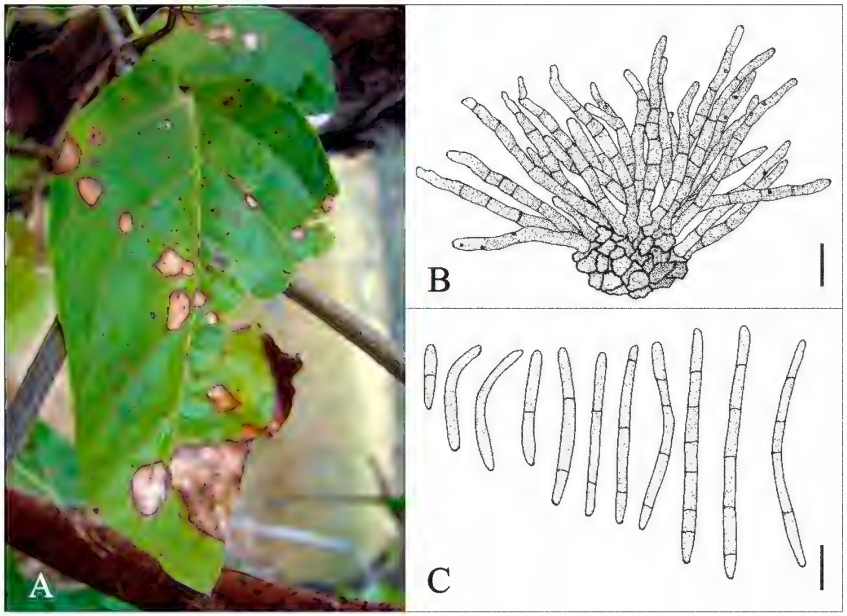

Plate 5. a: *Passalora macfadyenae* on *Macfadyena unguis-cati*: necrotic symptoms on infected leaves. b–c: *Passalora macfadyenae* (VIC 30480): b. conidiophore fascicle on stroma; c. conidia (straight to slightly curved). Bars = 10 µm.

slightly curved, 16–114 × 3–6 µm, 0–8 septate, unbranched, brown, smooth. Conidiogenous cells polyblastic, integrated, mostly cylindrical, 5–17.5 × 3.5–5 µm, brown. Conidiogenous loci conspicuous, 1–3 per conidiogenous cell, 1–2.5 µm, thickened and darkened. Conidia dry, solitary, cylindrical-obclavate, straight to slightly sinuous, 31.5–114 × 3–4.5 µm, base obconically truncate, apex rounded, 1–12 septate, hilum thickened and darkened, subhyaline to olivaceous, smooth.

Additional material examined: **BRAZIL. São Paulo**, on *Macfadyena unguis-cati*, 25 July 2004, OLPereira (VIC 30487).

Comments: No *Passalora* species are known to associate with *Macfadyena* taxa. Ten *Passalora* species are known to attack *Bignoniaceae* representatives, but all are dissimilar to the fungus collected on *M. unguis-cati*. Conidial sizes are useful in separating *P. macfadyenae* from many of the similar *Passalora* species of (Table 1). *Passalora tecomariae* Crous & B. Sutton and *P. tabebuiae-ochraceae* Inácio & Dianese differ from the new species in wider conidia, *P. tabebuiae* (J.J. Muchovej & F.A. Ferreira) U. Braun & Crous has narrower conidia, and *P. adenocalymmatis* U. Braun & Crous, *P. arrabidaeae* (Chupp & Viégas) Crous et al., and *P. pyrostegiae* (Viégas) U. Braun & Crous have longer conidia, smaller

Table 1. Conidial size of *Passalora* species recorded on members of the *Bignoniaceae*.

Species	Conidia (µm)	References
P. adenocalymmatis	35–150 × 3–4.5	Chupp (1954), as "*Cercospora adenocalymmae*"
P. arrabidaeae	35–150 × 3.5–5	Chupp (1954), as *Cercospora arrabidaeae*
P. catalpae	40–120 × 2.5–4.5	Chupp (1954), as *Cercospora catalpae*
P. catalparum	35–125 × 3.5–6	Chupp (1954), as *Cercospora catalparum*
P. leprosa	30–80 × 3.5–6	Chupp (1954), as *Cercospora leprosa*
P. macfadyenae	31.5–114 × 3–4.5	This publication
P. markhamiae	16–92.5 × 2.5–55.5	Liu & Guo (1982)
P. pyrostegiae	25–170 × 3–4.5	Chupp (1954), as *Cercospora pyrostegiae*
P. tabebuiae	(6–)8–30(–34) × 2.5–3	Muchovej & Ferreira (1981)
P. tabebuiae-ochraceae	31–75 × 5–8	Inácio & Dianese (2006)
P. tecomariae	35–90 × 5–6	Crous & Sutton (1997)

or absent stromata, and shorter conidiophores. *Passalora caltaparum* (Chupp) U. Braun & Crous has longer conidia, superficial hyphae, and solitary shorter conidiophores. *Passalora markhamiae* (X.J. Liu & Y.L. Guo) U. Braun & Crous has smaller stromata and shorter conidiophores and conidia. *Passalora leprosa* (Speg.) U. Braun is easily distinguished by having much larger stromata (300–500 µm diam), longer conidiophores (40–150 µm), and shorter conidia (30–80 µm). Additionally, *P. catalpae* (Chupp) U. Braun & Crous has smaller (<50 µm) or no stromata, longer (≤ 125 µm) conidiophores that are geniculate and rarely septate, and conidia that are acicular-shaped, indistinctly septate, and longer (40–120 µm) than those of *P. macfadyenae*. *Passalora macfadyenae* causes severe necrosis and significant defoliation of its host in the field (Plate 5a) and appears to have good potential for use in classical biological control of *M. unguis-cati*.

Pseudocercospora unguis-cati (Speg.) U. Braun, Mycotaxon 51: 49. 1994. Plate 6

Lesions on living leaves, amphigenous, subcircular, well delimited, infected tissue initially dark brown surrounded by a pale brown halo, becoming grayish centrally with a narrow dark brown outer rim at the periphery, 3–35 mm diam., coalescing and leading to necrosis of extensive areas of leaves. Internal mycelium indistinct. External mycelium absent. Stromata absent or small and composed of only a few substomatal swollen cells, pale brown. Conidiophores hypophyllous emerging through stomata, solitary or forming loose fascicles of few conidiophores, straight to slightly curved, sometimes restricted to the conidiogenous cell, cylindrical, 11–50 × 2.5–5 µm, 0–2 septate, unbranched, light brown, smooth, with inconspicuous scars. Conidiogenous cells terminal, holoblastic, mostly cylindrical, 10–17.5 × 2.5–5, pale brown. Conidiogenous loci inconspicuous, flat, up to 2.5 µm, unthickened, not darkened. Conidia dry,

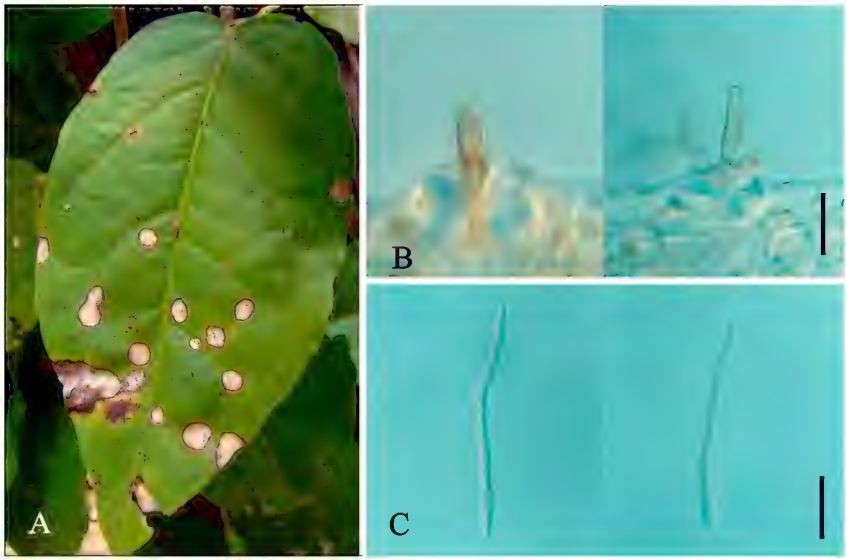

Plate 6. A. *Pseudocercospora unguis-cati* on *Macfadyena unguis-cati*: necrotic symptoms on infected leaves. B–C. *Pseudocercospora unguis-cati* (VIC 26575): B. conidiophores emerging through stoma; C. conidia. Bars = 10 µm.

solitary, acicular-obclavate, straight to slightly curved, 48–119 × 3.5–7 µm, base truncate, apex rounded, 0–6 septate, hilum unthickened, not darkened, pale brown, smooth.

Material examined: **BRAZIL**, **Minas Gerais**, on *M. unguis-cati*, 18 July 2004, OLPereira (VIC 26575); 18 May 2004, OLPereira (VIC 30479, 30481, 30482); 15 February 2005, OLPereira (VIC 30488); 2 February 2005, OLPereira (VIC 30489); 3 February 2005, OLPereira (VIC 30490, 30491); 25 July 2011, MSilva & OLPereira (VIC 31758). **Paraná**, on *M. unguis-cati*, 18 July 2004, OLPereira (VIC 26576). **São Paulo**, on *M. unguis-cati*, 26 July 2004, OLPereira (VIC 30486).

Comments: Only one cercosporoid species was previously known to occur on *Macfadyena* — *Pseudocercospora unguis-cati,* which was described from a specimen collected on *M. unguis-cati* in Argentina (Braun 1994). There are numerous *Pseudocercospora* species on hosts belonging to the *Bignoniaceae* (Crous & Braun 2003, Inácio & Dianese 2006). The survey in Brazil yielded two cercosporoid fungi that clearly belonged to the genera *Passalora* (see above) and *Pseudocercospora.* These were compared with the species described on *Bignoniaceae.* The *Pseudocercospora* specimen has morphological features that place it well within *P. unguis-cati* as described by Braun (1994). This is the first report of *P. unguis-cati,* originally described from Argentina, on *M. unguis-cati* in Brazil. *Pseudocercospora unguis-cati* causes necrotic spots on *M. unguis-cati*

(Plate 6a) that may be severe and should be further evaluated as a potential biocontrol agent.

Prospodium macfadyenae Meir. Silva, O.L. Pereira & R.W. Barreto, **sp. nov.**

MycoBank 561617 Plate 7–8

Differs from *Prospodium stizophylli* in larger (31.25–37.5 µm) teliospores, basket-like sori, and no mesospores and from *P. perornatum* in longer (47.5–55 µm) teliospores, larger (22.5–30 µm) urediniospores, and paraphyses within the sori.

Type: Brazil. Minas Gerais, on *Macfadyena unguis-cati*, 18 May 2004, OLPereira (VIC 30478, **holotype**).

Etymology: from the host genus name.

Lesions on living leaves, mainly on leaf veins but occasionally also widely distributed over the lamina, starting as chlorotic dots becoming pale brown centrally with well defined brown borders that later become dark brown to black and necrotic adaxially, appearing as black dots distributed within chlorotic areas, abaxially, of variable shape, 0.5–1.5 mm, leading to premature leaf fall. Internal mycelium intercellular 2.5–5 µm diam., branched, septate. Aecia unknown. Spermogonia epiphyllous, type 7, subepidermal, erumpent, in groups, conical, 16–38 × 6–19 µm, reddish brown. Uredinia and telia hypophyllous, in small scattered groups, basket-like sori, emerging through stomata, 152–221 µm diam., peripheral paraphyses 22.5–100 µm long, 5–12.5 µm wide, curved, cylindrical, somewhat inflated at base, tapering towards the acuminate apex, aseptate, chestnut brown, smooth; inner paraphyses, numerous, filiform to cylindrical, straight, 25–47.5 × 2.5–5 µm, aseptate, hyaline, smooth. Urediniospores globose to broadly ellipsoid, 30–37.5 × 22.5–30 µm, aseptate, wall bilaminate, the outer layer 2–2.5 µm thick, pale yellow, echinulate, inner wall up to 2.5 µm thick, yellow, germ pores, two, equatorial. Teliospores pedicellate, ellipsoid, 47.5–55 × 31.25–37.5 µm, rounded at both ends, one-septate, not constricted at the septum, wall 2.5–4.5 µm thick, chestnut brown, echinulate, two-pored, one apical pore in upper cell and one pore next to septum in the lower cell; pedicels attached basally or slightly obliquely, cylindrical, 50–62.5 × 5–7.5 µm, hyaline, persistent, with four or five conspicuous branched lateral appendages formed submedianly.

Additional material examined: **BRAZIL. Minas Gerais**, 25 Jul 2011, MSilva & OLPereira (VIC 31757).

Comments: Two rust fungi have previously been described in association with *M. unguis-cati*: *Uropyxis rickiana* Magnus [=*U. reticulata* Cummins] and *Prospodium stizophylli* H.S. Jacks. & Holw. (Cummins 1939; Carvalho Jr. et al. 2002, Hernández & Hennen 2003). *Uropyxis rickiana* is known only on *M. unguis-cati* from Argentina and Brazil (Hernández & Hennen 2003, Hennen et al. 2005) and *P. stizophylli* on *M. unguis-cati* and *Stizophyllum perforatum*

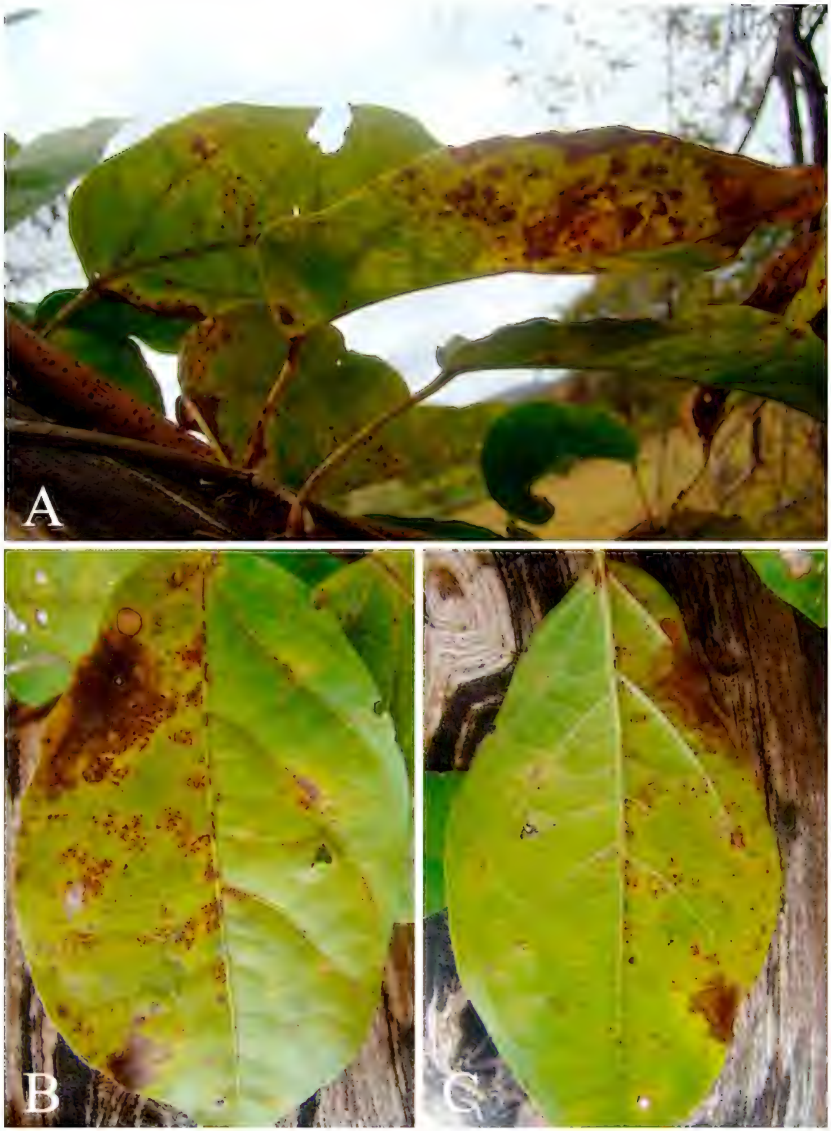

Plate 7. *Prospodium macfadyenae* on *Macfadyena unguis-cati*: A. Rust symptoms on infected plant; B. detail on adaxial leaf surface; C. detail on abaxial leaf surfaces.

(Cham.) Miers from Brazil (Carvalho Jr. et al. 2002). *Uropyxis* species differ from *Prospodium* species by having two pores on each teliospore cell instead of only one. The species collected during the present study clearly belongs to *Prospodium*. *Prospodium macfadyenae* differs from *P. stizophylli* by having

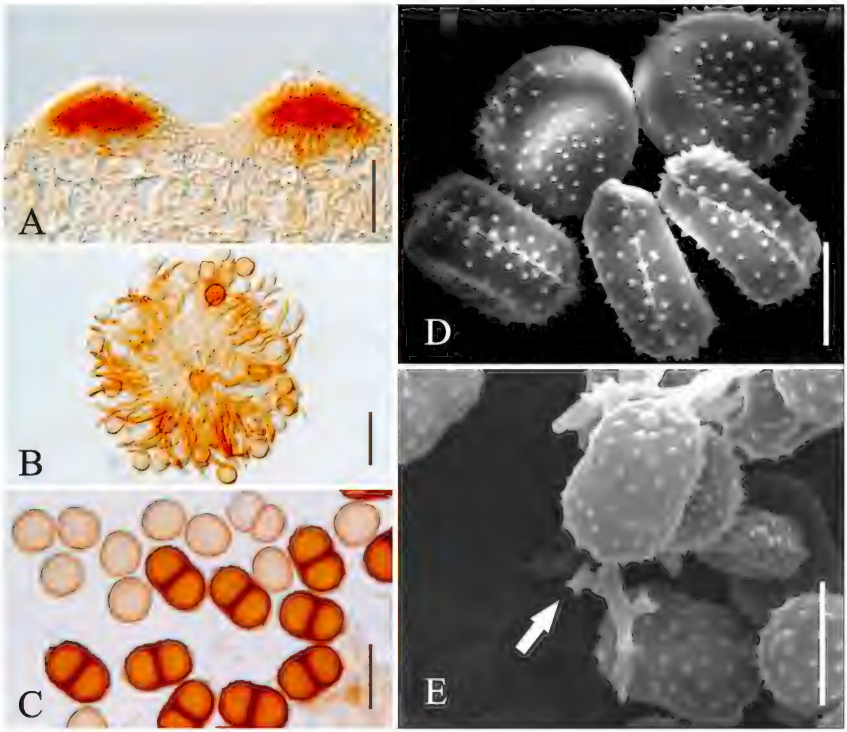

PLATE 8. *Prospodium macfadyenae* (VIC 30478): A. type 7 spermogonium; B. basket-like sori (squash-mount); C. echinulate urediniospores intermixed with pedicellate 2-celled teliospores. Scanning electron micrograph (SEM) of spores: D. surface view of echinulate urediniospores; E. mature teliospores (note branched appendages on pedicels – arrowed). Bars: A = 10 µm; B = 50 µm; C = 30 µm; D = 10 µm; E = 20 µm.

longer, broader teliospores and the absence of mesospores. *Prospodium stizophylli* also does not have basket-like sori, does not produce uredinia, and has a pseudoreticulate ornamentation on its teliospores. *Prospodium macfadyenae* is morphologically most similar to *P. perornatum* Syd. (Cummins 1940, Hennen et al 2005), known only on *Tabebuia* spp. from Argentina and Mexico (Hernández & Hennen 2003), but differs from the latter by having larger urediniospores, longer teliospores that are not constricted at the septum, and the presence of paraphyses within the sori in addition to thick walled incurved peripheral paraphyses.

Prospodium macfadyenae causes significant damage to the foliage of *M. unguis-cati* (PLATE 7), often leading to significant defoliation of its host, and belongs to a group of fungi that includes several highly successful classical biological control agents of weeds. Rust fungi generally have a high level of host-

specificity (and thus fit well into the safety requirements needed for classical introductions). Therefore, we regard the prospects for its use in biocontrol of *M. unguis-cati* as very good. Although *U. rickiana* has not been collected during the surveys in southern and southeastern Brazil, it is likely that this species also has potential as a biological control agent in warmer equatorial areas where *M. unguis-cati* occurs as an invasive plant. It has the same favorable features described for *P. macfadyenae* and causes a severe disease on its host producing conspicuous galls on stems as illustrated in Hernández & Hennen (2003).

Ideally, further investigations involving additional surveys and a more detailed evaluation of the potential biocontrol agents already uncovered are needed. These could yield additional candidates for use in classical biological control of *M. unguis-cati* and help pave the way towards introducing *Pseudocercospora unguis-cati*, *Passalora macfadyenae* and *Prospodium macfadyenae* into exotic situations where invasions by cat's claws are under way, contributing to the mitigation of the environmental damage caused by this important invasive weed.

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Typification of *Lecanora expallens* and *L. expallens* var. *conizaea* (*Lecanorales, Ascomycota*)

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Abstract — Lectotypes are selected for *Lecanora expallens* and *L. expallens* var. *conizaea* from the collection in H-ACH. Characteristic of the species based on the type collection is provided.

Key words — *Lecanoraceae,* lichenized fungi, lichen metabolites

Introduction

Lecanora expallens is a well known, common and widely distributed sorediate lichen species, reported from many regions of the world (e.g. Tønsberg 1992, Pišút et al. 1996, Motiejūnaitė 1999, Diederich & Sérusiaux 2000, Śliwa & Wetmore 2000, Hafellner & Türk 2001, Coppins 2002, Fałtynowicz 2003, Kurokawa 2003, Aptroot et al. 2004, Ryan et al. 2004, Santesson et al. 2004, Galloway 2007). It was included in the taxonomical revision of the *Lecanora varia* group in North America (Śliwa & Wetmore 2000) and in the recent lichen flora of the Greater Sonoran Desert Region (Ryan et al. 2004) as well. The species was also discussed by Printzen (2001) in relation to *L. confusa* Almb., a taxon which is supposed to be an esorediate counterpart of *L. expallens* and considered by some authors as doubtfully distinct from the latter (see Śliwa & Wetmore 2000).

During my research on sorediate crustose lichens, several sterile and fertile specimens of *L. expallens* were investigated. After the examination of all available literature it became clear that although *L. expallens* was a common subject of several studies, no type specimen of the species has ever been cited and actually the identity of the species has remained obscure. That prompted the examination of the type collections of *L. expallens*, including *L. expallens* var. *conizaea*. The aim of this paper is to present the typification of *L. expallens* and *L. expallens* var. *conizaea*.

Material & methods

Type material from BM-ACH and H-ACH was studied, with additional specimens from BM, KRAM and UGDA examined for comparison. Lichen substances were studied by thin-layer chromatography (TLC) in solvents A and C according to the methods of Culberson & Kristinsson (1970) and Orange et al. (2001).

Taxonomy

Lecanora expallens Ach., Lichenogr. Univer.: 374. 1810.

TYPE: Gallia, (on wood) Du Four 99 (H-ACH 1267, upper left hand specimen – **lectotype designated here**).

= *Lecanora expallens* var. *conizaea* Ach., Lichenogr. Univer.: 374. 1810.

≡ *Lecanora conizaea* (Ach.) Nyl. ex Cromb., J. Linn. Soc., Bot. 15: 440. 1876.

TYPE: Anglia (on bark of pine) Turner (H-ACH 1267, right hand specimen – **lectotype designated here**).

THALLUS crustose, grey green, episubstratal, entirely sorediate, without any delimited soralia (marginal part not preserved in the collected specimen); SOREDIA farinose; APOTHECIA lecanorine, sessile, flat to slightly convex, numerous; DISC brown, delicately pruinose, pruina farinose; THALLINE MARGIN well developed, persistent, raised above the disc, sorediate, concolorous with the thallus, cortex not seen; AMPHITHECIUM with two different types of crystals, one present in the outer part, yellowish in polarized light, K soluble and N insoluble, the other in the inner part, more whitish in polarized light, K insoluble and N soluble; PARATHECIUM well visible, up to 40 µm wide, with substance (amorphic?) yellow in polarized light, K soluble, N insoluble; EPITHECIUM brownish, granulose, granules coarse, yellow in polarized light, K soluble and N mostly insoluble (or slowly soluble); PARAPHYSES simple, up to 2 µm wide, not capitate; ASCOSPORES 8 in ascus, non-septate, hyaline, narrowly (9–14 × 4–5 µm) or broadly (11 × 6–6.5 µm) ellipsoid.

CHEMISTRY — Usnic acid (+), zeorin (+), thiophanic acid (+), xanthone similar to arthothelin (+), 'expallens unknown' sensu Tønsberg (1992) and trace of unidentified xanthone (±, both only in the lectotype of *L. expallens* var. *conizaea*) were detected in the studied type specimens. That agrees with the chemistry reported by Tønsberg (1992), Śliwa & Wetmore (2000), Printzen (2001) and Ryan et al. (2004).

COMMENTS — According to Kärnefelt & Thell (2007), the main part of Acharius collection is placed at the University of Helsinki as H-ACH. Less comprehensive (but still large) collections are also present in the Museum of Evolution in Uppsala (UPS-ACH), the Natural History Museum in Stockholm (S), and the Natural History Museum, London (BM-ACH) (see also Vondrák & Vitikainen 2008). Specimens of *L. expallens* were found only in H and BM.

The collection in H-ACH contains five specimens, all associated at least with the country name and some also with further information. Two of them

correspond to the protologue in Acharius (1810), therefore the collection in H-ACH is suitable for the typification. In BM-Ach the specimen consists of several pieces of wood covered with thallus of *L. expallens*, but no details of locality and collector are provided.

Acharius (1810) cited 'Habitat ad truncos Salicinos Galliae. *Du Four. Persoon.*' Both those collections are present in H-ACH; however, only Du Four's specimen belongs to *L. expallens* in the currently accepted sense, and that one is selected in this paper as a lectotype to preserve the current concept of the species. The second specimen represents *Pertusaria coccodes* (Ach.) Nyl. (norstictic and connorstictic acids were detected by TLC). Both specimens have also been examined and annotated by O. Almborn in 1963, but his results have never been published (O. Vitikainen, pers. information).

Acharius (1810) cited three specimens of *L. expallens* var. *conizaea*: 'Habitat in cortice Pini ac ligno Quercus vetusto Angliae, *Turner*; Helvetiae, *Schleicher.*' In H-ACH only one specimen annotated as *L. expallens* var. *conizaea* exists; it is on piece of bark of pine from 'Anglia' and is here selected as lectotype. This name (as *L. conizaea*) was often misapplied for *L. conizaeoides* Nyl. ex Cromb. and *L. strobilina* (Spreng.) Kieff. (Laundon 1976, 2003). However, as the type corresponds morphologically and chemically with the lectotype of *L. expallens*, *L. expallens* var. *conizaea* is confirmed as a synonym of *L. expallens*, which was already mentioned by Laundon (2003).

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The morphology of *Ganoderma* species with a laccate surface

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Abstract — A discussion on the importance of morphological features in the taxonomy of *Ganoderma* subg. *Ganoderma* is presented. More than 500 specimens of 37 species, including 33 types, were checked: *G. argillaceum*, *G. atkinsonii*, *G. boninense*, *G. capense*, *G. chonoides*, *G. colossus*, *G. concinnum*, *G. corrugatum*, *G. curtisii*, *G. dorsale*, *G. elegantum*, *G. longistipitatum*, *G. mexicanum*, *G. multicornum*, *G. multiplicatum*, *G. nevadense*, *G. nitidum*, *G. oerstedii*, *G. orbiforme*, *G. oregonense*, *G. parvulum*, *G. perturbatum*, *G. perzonatum*, *G. praelongum*, *G. pulverulentum*, *G. ravenelii*, *G. resinaceum*, *G. sequoiae*, *G. sessile*, *G. sessiliforme*, *G. simulans*, *G. stipitatum*, *G. subfornicatum*, *G. subincrustatum*, *G. vivianimercedianum*, *G. weberianum*, and *G. zonatum*. Color of the context, resinous deposits, structure of the basidiospores, and protuberances of the pileipellis cells are among the most important features for characterization of the species.

Key words — *Basidiomycota*, *Ganodermataceae*

Introduction

Ganoderma P. Karst. is characterized by its double-walled, colored basidiospores with a truncate or subacute apex and ornamented exosporium. The genus includes species with both dull [subg. *Elfvingia* (P. Karst.) Imazeki] and laccate pilei (subg. *Ganoderma*). The pileipellis has been differently characterized by different authors: (1) Furtado (1965a) refers to a derm of the palisadoderm type in *Elfvingia* and layer of cells in a hymeniderm in *Ganoderma,* while Steyaert (1980) refers to a trichoderm in *Elfvingia* and "hymenioderm" in subg. *Ganoderma.* Clémençon (2004) used "crustohymeniderm" for subg. *Ganoderma*, so following his definition, the *Elfvingia* pileipellis is a crustotrichoderm, because it is also incrusted with a resinous matter. Although phylogenetic analyses (Moncalvo 2000, Hong & Jung 2004) support the

monophyly of *Ganoderma* and its classification in two subgenera, there are many problems in the resolution below subgeneric level.

The micromorphological structures of some species or groups in *Ganoderma* have been studied by, among others, Patouillard (1889), Murrill (1902), Haddow (1931), Heim (1962), Furtado (1965a,b, 1967), Steyaert (1967a,b, 1972, 1975, 1977), Pegler & Young (1973), Corner (1983), and Adaskaveg & Gilbertson (1988). More recently, Gottlieb & Wright (1999) made a detailed study of the basidiospores under the Scanning Electron Microscope (SEM) in an attempt to solve some of the inter-specific problems. However, these studies have been restricted to a rather limited number of species and few used the pileipellis cells as a critical character in their species concepts.

Through the study of more than 500 specimens (including 33 types) mainly from Africa, Brazil, Costa Rica, Mexico, and USA, we analyzed the morphological variation of structures in *Ganoderma* subg. *Ganoderma* with the following aims: 1) to study in detail structures currently and potentially useful in taxonomy and phylogenetic analyses and 2) to probe the utility of light microscopy in defining basidiospore pillars.

Materials & methods

Specimens examined

More than 500 specimens from the herbaria BPI, BR, ENCB, FH, H, IBUG (including collections by the first author), INB, K, NY, O, PC, SP, UPS, and XAL were examined (acronyms follow Holmgren et al. 1990). These specimens comprised 37 species, which are listed in Table 1, which includes one representative specimen for each species.

Macro and micromorphological observations and identification

The following macromorphological characters were described for each basidiome: length, width, thickness, adhesion to the substrate, general shape, consistency, weight; pileus shape, surface consistency, color; context thickness, stratification, color, resinous deposits; pore form, number per mm, consistency, color; tube length, stratification, color. Macrochemical reactions used 10% KOH. In addition to the traditional microcharacters, descriptions include: basidiospore apex, disposition and size of basidiospore pillars, and pileipellis cell size, shape, and incrustations. Color references follow Kornerup & Wanscher (1963). Microscopical observations were made from basidioma tissues mounted in 10% KOH and Melzer's reagent, with Congo red, phloxine, and cotton blue additionally used. Basidiospore shape is determined from the Q ratio (l/b; Bas 1969: 320–321) of 20 randomly selected basidiospores. Only mature pileipellis cells were described. Microscopical observations were made using a 100× oil-immersion objective in Zeiss K7 and Zeiss Axioskop 40 microscopes. The inter-walled basidiospore pillars were measured using Axio Vision 4 software with the Zeiss Axioskop 40 microscope. Most morphological terms follow Vellinga (1998), Furtado (1965a), and Clémençon (2004). The pileipellis description follows the concepts of Clémençon (2004).

Table 1. Species of *Ganoderma* subg. *Ganoderma* with representative collections.

Species	Specimen
G. argillaceum Murrill	F.S. Earle 658 (lectotype, NY)
G. atkinsonii H. Jahn et al.	without data (O)
G. boninense Pat.	C. Wright s.n. (lectotype, K)
G. capense (Lloyd) Teng	P. van der Bijl s.n. (lectotype, NY)
G. chonoides Steyaert	without data (holotype, BR)
G. colossus (Fr.) C.F. Baker	A.S. Oersted s.n. (lectotype, UPS)
G. concinnum Ryvarden	L. Ryvarden 16840 (holotype, O)
G. corrugatum Steyaert	R.L. Steyaert 45061 (holotype, BR)
G. curtisii (Berk.) Murrill	P.O. Schallert s.n. (F1119720)
G. dorsale (Lloyd) Torrend	R.J. Rick s.n. (holotype, BPI)
G. elegantum Ryvarden	Ryvarden 44573 (holotype, O)
G. longistipitatum Ryvarden	L. Ryvarden 40588 (holotype, O)
G. mexicanum Pat.	without data (lectotype, FH)
G. multicornum Ryvarden	G.J. Samuels s.n. (holotype, O)
G. multiplicatum (Mont.) Pat.	R. Maziero s.n. (SP250605)
G. nevadense Murrill	C.F. Baker 1489 (lectotype, NY)
G. nitidum Murrill	P. Wilson 607 (lectotype, NY)
G. oerstedii (Fr.) Murrill	A.S. Oersted s.n. (neotype, UPS)
G. orbiforme (Fr.) Ryvarden	A. Afzelius s.n. (holotype, UPS)
G. oregonense Murrill	J.E. Kirkwood s.n. (lectotype, NY)
G. parvulum Murrill	C.L. Smith s.n. (lectotype, NY)
G. perturbatum (Lloyd) Torrend	R. Rick s.n. (holotype, BPI)
G. perzonatum Murrill	F.S. Earle 309 (lectotype, UPS)
G. praelongum Murrill	F.S. Earle & W.A. Murrill 536 (lectotype, NY)
G. pulverulentum Murrill	W.E. Broadway s.n. (lectotype, NY)
G. ravenelii Steyaert	H.W. Ravenel 2936 (holotype, K)
G. resinaceum Boud.	J.L. Boudier s.n. (holotype, PC)
G. sequoiae Murrill	A. Eastwood 40 (lectotype, NY)
G. sessile Murrill	without data (lectotype, NY)
G. sessiliforme Murrill	E. & W.A. Murrill 392 (lectotype, NY)
G. simulans Wakef.	T.D. Maitland 556 (isotype, O)
G. stipitatum (Murrill) Murrill	C.L. Smith s.n. (lectotype, NY)
G. subfornicatum Murrill	M.E. Peck s.n. (lectotype, NY)
G. subincrustatum Murrill	F.S. Earle 176 (lectotype, NY)
G. vivianimercedianum M.G. Torres	E. Bastidas-Varela s.n. (holotype, ENCB)
G. weberianum (Bres. & Henn. ex Sacc.) Steyaert	A. Milanez & D. Altimari s.n. (SP61099)
G. zonatum Murrill	L.M. Underwood s.n. (lectotype, NY)

Specimens were generally determined after comparing with the type or types of related species, but the keys of Bazzalo & Wright (1982) and Ryvarden (2004) and the descriptions of Steyaert (1972) and Corner (1983) were also used.

Results

The features proposed here are discussed below and are being used to describe and key *Ganoderma* species from Brazil and Mexico (Torres-Torres et al., papers in preparation).

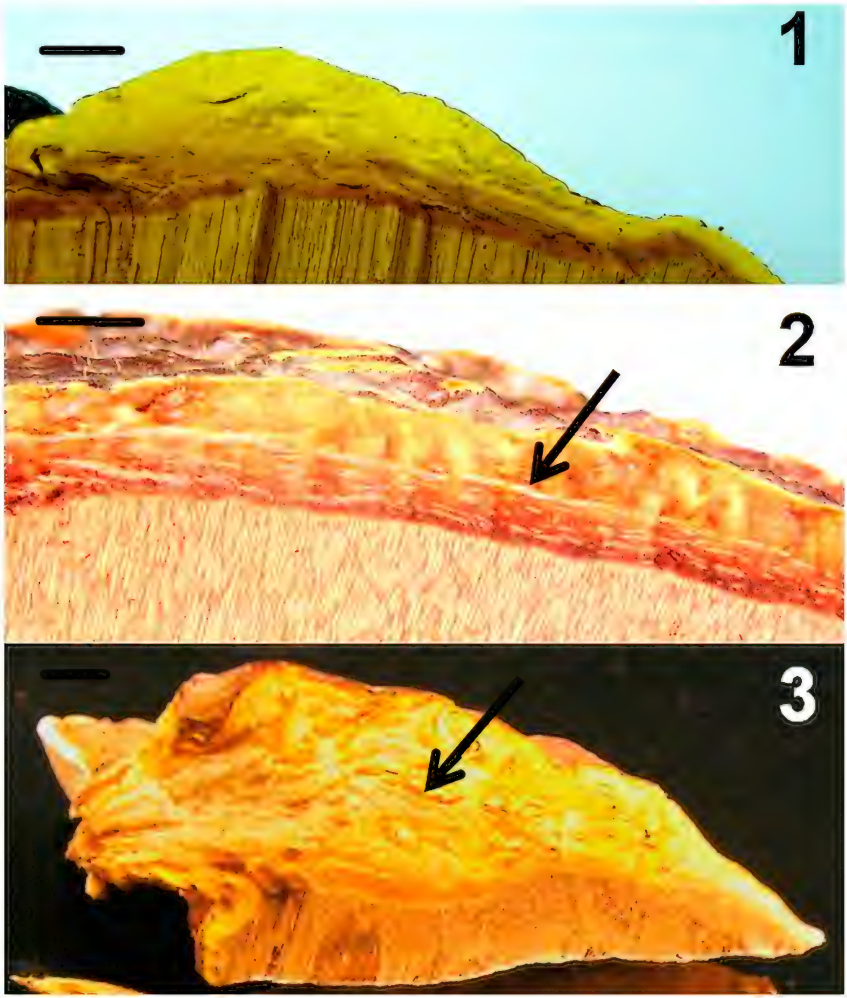
Macromorphological features

Basidiome shape and substrate attachment are of rather restricted value for taxon delimitation; however, basidiome consistency, weight, and thickness as well as the presence and attachment of the stipe proved, in some cases, to be valuable for identification. *Ganoderma atkinsonii*, *G. colossus*, *G. nevadense*, and *G. oregonense* have light, spongy basidiomata, while *G. zonatum* and *G. pulverulentum* have light, woody-corky basidiomata. *Ganoderma mexicanum*, *G. sessile*, *G. sessiliforme*, and *G. subincrustatum* have relatively light, woody basidiomata, while *Ganoderma oerstedii* has heavy, woody basidiomata. *Ganoderma colossus*, *G. oregonense*, *G. oerstedii*, *G. sessile*, and *G. zonatum* are generally robust, while *G. mexicanum*, *G. parvulum*, *G. sessiliforme*, and *G. weberianum* have slender basidiomata.

The more important features of the pileus context for diagnosing species are: (1) consistency, (2) structure, and (3) presence of resinous deposits. Only three types of context consistency are recognized. Two — soft-spongy and fibrous — are easy to distinguish, but there is an intermediate state between soft and fibrous that is difficult to identify. In this study, the third type was termed "fibrous-spongy." *Ganoderma colossus* and *G. oregonense* have soft-spongy context, *G. sessiliforme* has fibrous context, and *G. resinaceum* has fibrous-spongy context.

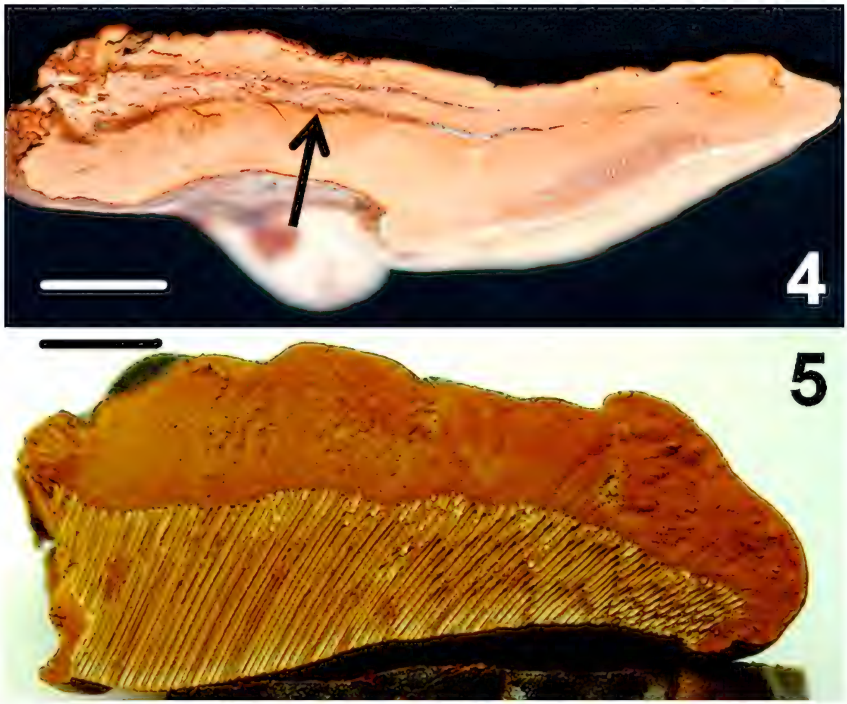
The context structure is classified as (1) duplex, (2) not fully homogeneous, or (3) homogeneous. In the duplex context there is an abrupt change, with two separate, contrasting colors, the upper generally light-colored and the lower generally darker close to the tubes, although shades may vary to some extent. This context type is present in *G. concinnum*, *G. curtisii*, *G. dorsale*, and *G. sessile* (Fig. 1). Not fully homogeneous is used when there is an evident color difference between the upper and lower parts but without abrupt color changes, like in *G. oerstedii* (Fig. 2), *G. perturbatum*, *G. perzonatum*, *G. weberianum* (Fig. 3), while a homogeneous context has only one color, as in *G. parvulum* and *G. resinaceum* (Figs. 4–5). Contexts with an occasional very thin darker line just above the tubes that is absent in some specimens are considered as homogeneous. The context can contain resin-like deposits (hard and brittle, dull or shiny) that often they form continuous bands (here "resinous bands," see *G. oerstedii* and *G. parvulum* in Figs. 2, 4) or discrete bodies (here "resinous incrustations," see *G. weberianum,* Fig. 3).

Some authors (Patouillard 1889, Moncalvo 2000, Seo & Kirk 2000) have dismissed the stipe as a character of no taxonomic value, because some sessile species can develop a stipe in the laboratory resulting from varying oxygen conditions. Nevertheless, the stipe is a useful character, with a "true" stipe defined as one > 5 cm and with tissues differentiated from the pileus context. Some species develop a generally short (<5 cm) pseudostipe that is merely a



Figs. 1–3. *Ganoderma* macromorphology. **1**. Duplex context *(G. sessile)*. **2**. Not completely homogeneous context *(G. oerstedii)*; continuous bands of the resin-like deposits (arrowed). **3**. Not completely homogeneous context *(G. weberianum)*; discrete bodies of the resin-like deposits (arrowed). Bar = 2 cm.

continuation of the pileus and which Steyaert (1980) termed a "pedicel" and we call a "substipe." Species such as *G. curtisii*, *G. concinnum*, *G. dorsale*, and *G. perturbatum* always present a true stipe, while *G. pulverulentum* and *G. weberianum* can develop a substipe dependent upon environmental and nutritional conditions.

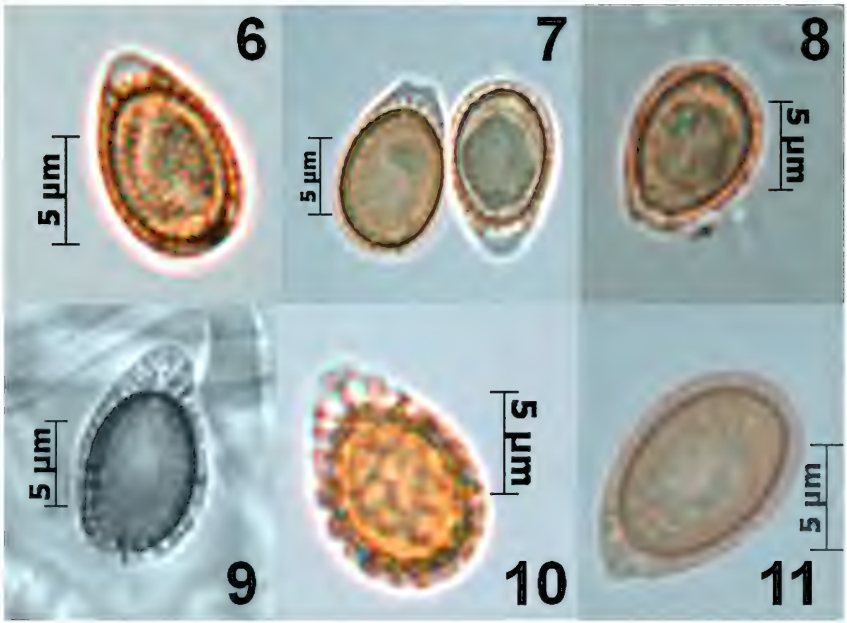


Figs. 4–5. *Ganoderma* macromorphology. **4.** Homogenous context *(G. parvulum)*; continuous bands of the resin-like deposits (arrowed). **5.** Homogeneous context *(G. resinaceum)*; note the absence of resin-like deposits. Bar = 2 cm.

Micromorphological features

Basidiospores. Following Clémençon's (2004) terminology, all *Ganoderma* basidiospores have a thin, smooth or rugose, hyaline to reddish-yellow perisporium; an ornamented colored exosporium with pillars, and a thick reddish-brown or yellowish-brown endosporium. Using the Q ratio categories (Bas 1969), most species have ellipsoid to elongate (= oblong) basidiospores, while *G. concinnum*, *G. dorsale* and *G. perturbatum* have distinctively widely ellipsoid to ellipsoid basidiospores and *G. ravenelii* and *G. zonatum* have oblong to cylindrical basidiospores. *Ganoderma* basidiospores have a cap in the apex (Figs. 6–7) that is obtuse and hyaline; after drying, the cap generally collapses and breaks, making the basidiospore apex truncate (Figs. 7–8). However, in some species where the cap is absent or very small, the basidiospores have a subacute apex, as in *G. atkinsonii*, *G. colossus*, *G. concinnum*, *G. dorsale*, *G. longistipitatum*, *G. oregonense,* and *G. perturbatum* (Figs. 9–11).

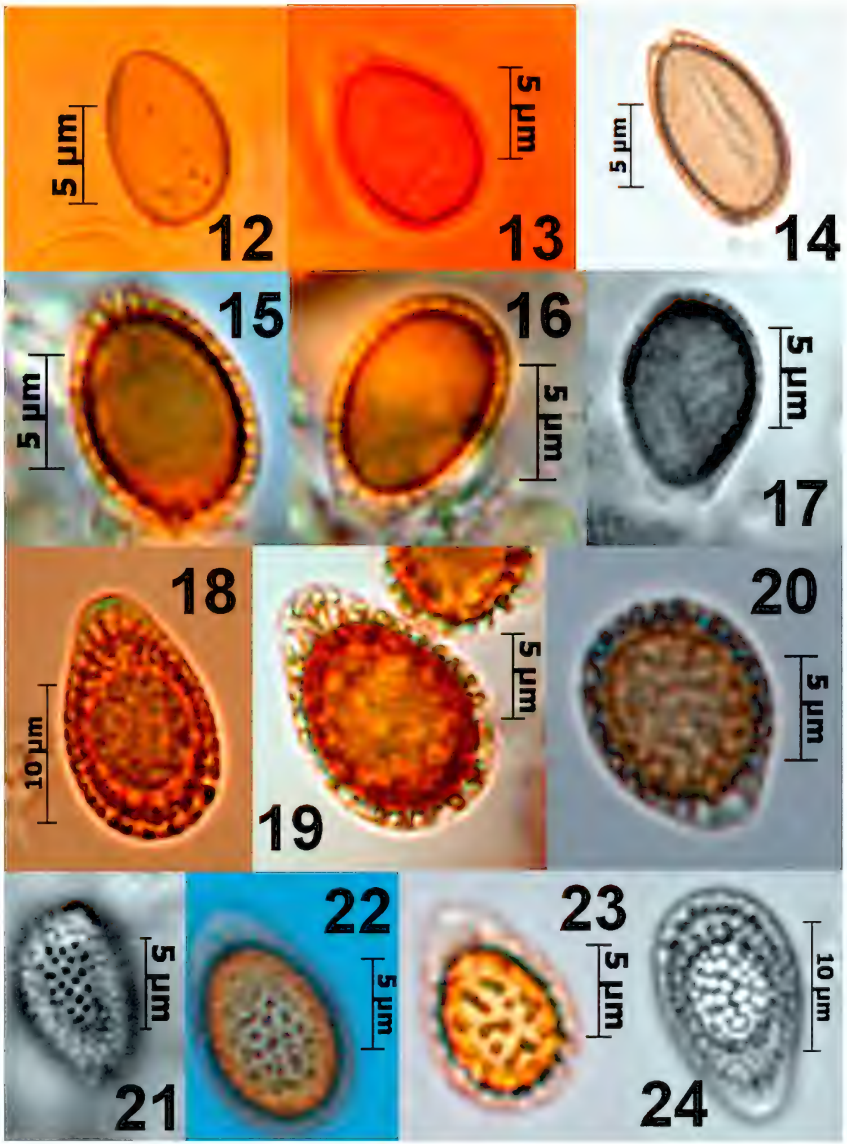
The basidiospore inter-walled pillars are thin to thick; thin (<0.3 μm) in *G. mexicanum*, *G. perzonatum*, *G. praelongum*, and *G. zonatum* (Figs.



Figs. 6–11. *Ganoderma* micromorphology. **6**. Basidiospores with cap *(G. multiplicatum)*. **7**. Basidiospore (right) with cap and (left) with truncate apex *(G. sessiliforme)*. **8**. Basidiospores with truncate apex *(G. chonoides)*. **9–11**. Basidiospores with subacute apex (9. *G. atkinsonii*; 10. *G. concinnum*; 11. *G. oregonense*).

12–14), intermediately thick (0.3–0.5 µm) in *G. corrugatum*, *G. curtisii*, *G. oerstedii*, *G. pulverulentum*, *G. resinaceum*, *G. sessiliforme*, *G. subincrustatum*, and *G. weberianum* (Figs. 15–17), and thick (0.5–0.7 µm) in *G. colossus*, *G. longistipitatum*, *G. oregonense*, and *G. perturbatum* (Figs. 18–20). The pillars can be free, sub-free, partially anastomosed, and reticulate (= anastomosed). "Free" pillars appear as dots on the basidiospore surface (e.g. *G. perzonatum*, fig. 21), while "sub-free" are free dots mixed with short anastomosed to shortly elongated structures (e.g. *G. elegantum*, fig. 22). "Partially anastomosed" is used when more than two pillars are grown together to form an irregular surface (e.g. *G. concinnum*, fig. 23), and "reticulate" is when the ornamentation forms an almost complete net (e.g. *G. colossus*, fig. 24).

Structure of the pilear cover. The pilear surface or pileipellis is formed by a cell layer similar to a hymeniderm, although in the majority of the species the cells do not originate from the same level as in the true hymeniderm. Clémençon (2004) defined the *Ganoderma* type of hymeniderm as a crustohymeniderm, which is characterized by thick-walled cells apically covered by a resinous



Figs. 12–24. *Ganoderma* micromorphology. **12–14**. Basidiospores with thin inter-walled pillars (12. *G. perzonatum*; 13. *G. praelongum*; 14. *G. zonatum*). **15–17**. Basidiospores with intermediate thick inter-walled pillars (15. *G. pulverulentum*; 16. *G. weberianum*; 17. *G. corrugatum*). **18–20**. Basidiospores with thick inter-walled pillars (18. *G. colossus*; 19. *G. longistipitatum*; 20. *G. perturbatum*). **21–25**. Disposition of the pillars in the basidiospores: 21. Free *(G. perzonatum)*; 22. Subfree *(G. elegantum)*; 23. Partially anastomosed *(G. concinnum)*; 24. Reticulate *(G. colossus)*.

matter. Cell sizes must be interpreted with care because they vary not only between species but also in the same specimen according to where the section has been made. Most species have club-like apical cells although there are species with branched or knobby cells besides the club shaped cells (Fig. 25).

The shape of the crustohymeniderm cells can be: (1) cylindrical (*G. perzonatum*), (2) almost cylindrical to narrowly clavate (*G. weberianum*), (3) widely clavate (*G. perturbatum*), and (4) clavate (most species). The cell wall is thick for all except *G. colossus*, where it is thin. A thick wall may be multi-stratified, related to the maturity state of the basidiomata; however, some species, like *G. perturbatum*, have an evidently multi-stratified wall. Some young pileipellis cells present secondary septa, more evident in the cells of the pileus periphery; furthermore, these peripheral cells tend to be lighter and shorter. Incrustations or granulations can also be present in the pileipellis. The incrustations are easily dissolved in KOH [e.g. *G. stipitatum*, fig. 27], while the granulations remain, because they are not alkali-soluble (e.g. *G. perzonatum*, fig. 26).

In some species the pileipellis cells have a transition from smooth club-like to antler-like cells. Protuberances and branches (as used in the descriptions below) differ in that "protuberances" are short projections or prolongations of the hyphal wall (generally≤ 8 μm long, located laterally or at the apex) that lack a lumen whereas "branches" may be short or large, apical or lateral but always possess a lumen. There are shape series in which the cell changes progressively, but a predominant shape is always observed (Fig. 25). Three large groups of crustohymeniderm cells might be found: entire (Figs. 25a, 26–28), lobulate-branched (Figs. 25b, 29–30), and branched (Fig. 31).

1) "Entire" crustohymeniderm cells are cylindrical, cylindrical with slightly wide apex, cylindrical to narrowly clavate, cylindrical with subcapitate apex, or narrowly clavate to widely clavate, generally lacking protuberances or branches, very occasionally with at most two, generally lateral protuberances, e.g. in *G. perturbatum*, *G. perzonatum*, *G. stipitatum*, and *G. sessiliforme* (Figs. 25a, 26–28).

2) "Lobulate-branched" crustohymeniderm cells may be subdivided into: a) clavate cells, with 1–3 round short thick protuberances and/or branches, but preserving the clavate shape (e.g., *G. nitidum*; *G. zonatum,* Fig. 25b); b) cells with no or 1-2 thick long branches and ≤ 7 short thick protuberances, where the clavate shape is generally lost (e.g., *G. oerstedii*; *G. subincrustatum,* Figs. 25c, 31; *G. multiplicatum,* Figs. 29–30).

3) "Branched" crustohymeniderm cells, with two types that generally lose the clavate shape: a) with 2-3 thin long branches with small protuberances (e.g., *G. orbiforme,* Fig. 25d) and b) the most striking, cells with many antler-like branches and small protuberances (e.g., *G. multicornum,* Fig. 25e).

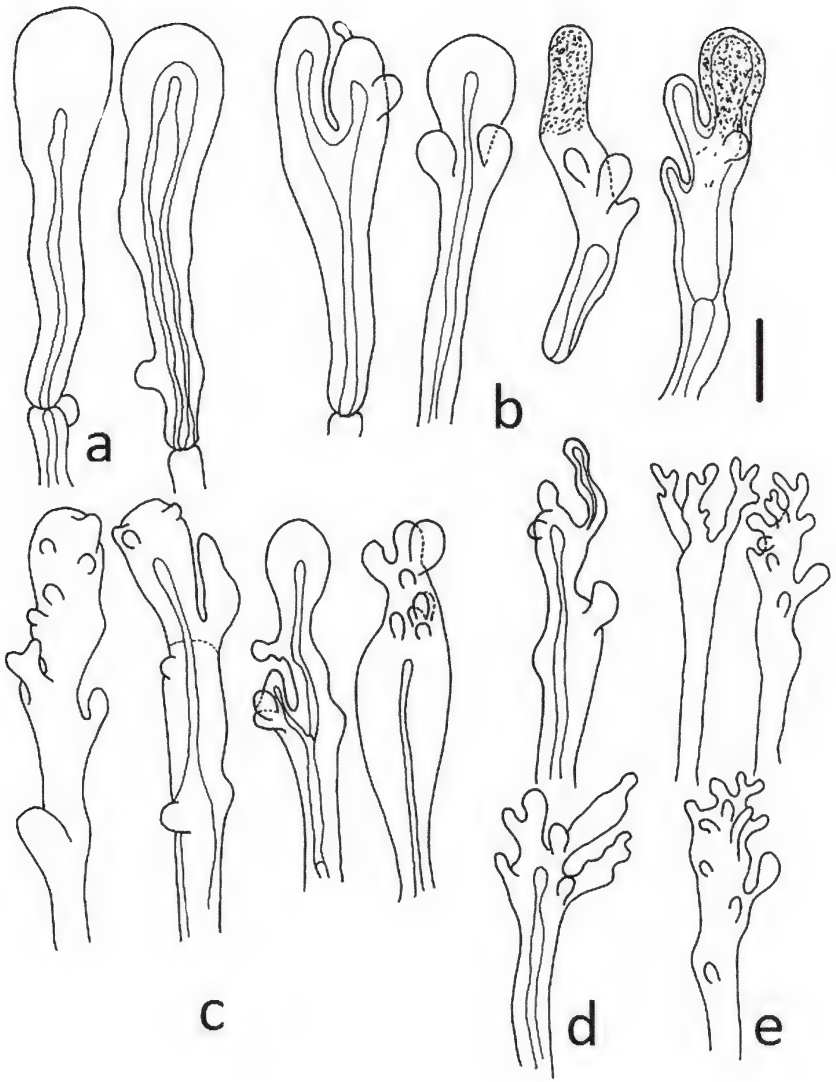


FIG. 25. *Ganoderma* crustohymeniderm cells. **a**. Entire *(G. perturbatum, G. sessile)*. **b**. Lobulate-branched *(G. nitidum, G. zonatum)*. **c**. Lobulate-branched with up to seven short and thick protuberances and 0 or 1–2 thick and long branches *(G. oerstedii, G. subincrustatum)*. **d**. Branched *(G. orbiforme)*. **e**. Antler-like *(G. multicornum)*.

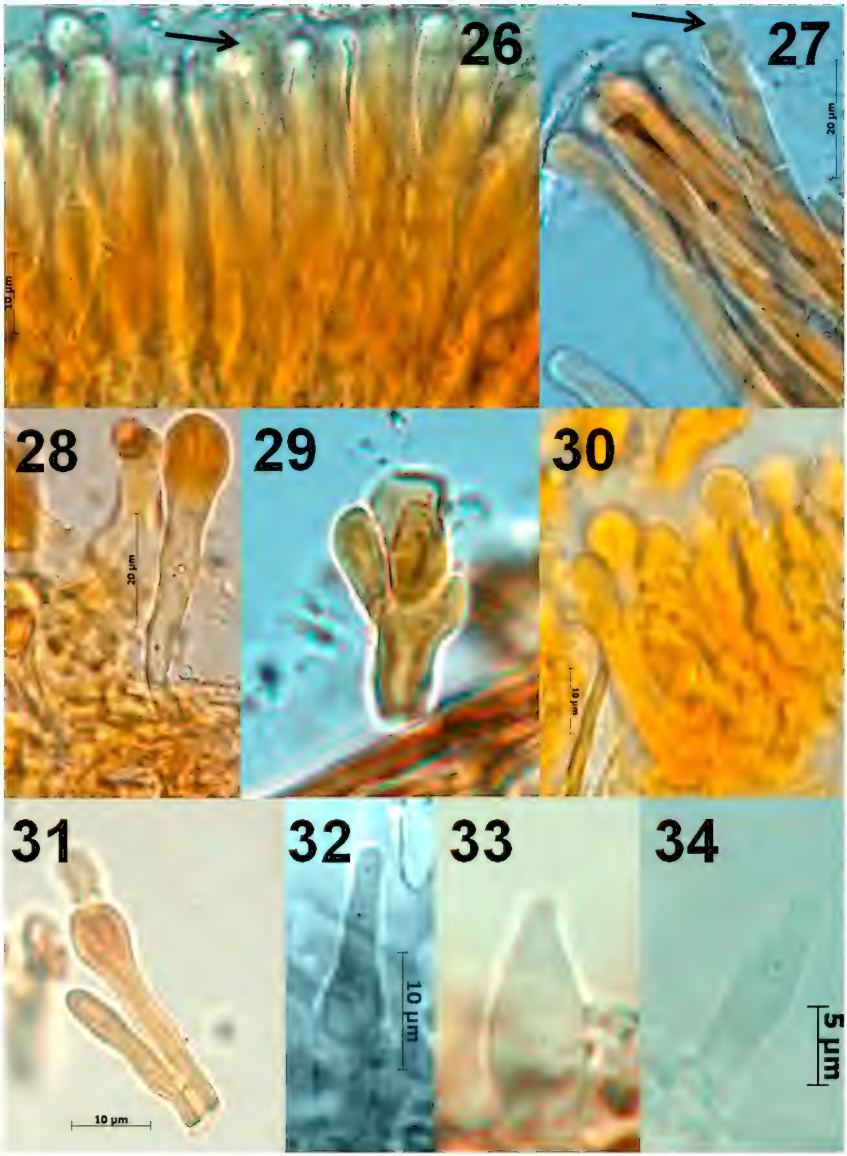
Hyphal system. In all species the hyphal system is di-trimitic, where generative and binding hyphae are generally difficult to observe. All generative hyphae have clamped septa and are hyaline to yellowish and (in almost all species) branched. Skeletal hyphae are of the arboriform type, thick-walled to solid, yellowish to yellowish-brown, apically branched; thin in the crustohymeniderm, wider in the context. Binding hyphae are generally thinner than skeletal hyphae, hyaline to yellowish-brown.

Cystidia. Vellinga (1998) defined them as sterile, differentiated, terminal elements in the hymenium. According with this definition *G. colossus*, *G. oregonense*, *G. perzonatum*, *G. pulverulentum*, *G. sequoiae*, and *G. stipitatum* have cystidia (Figs. 32–34), but they are difficult to observe.

Discussion

It is clear that species concepts vary in *Ganoderma* according to the author. Historically, *Ganoderma* taxa have been treated as species with great plasticity (e.g. *G. resinaceum* by Ryvarden, 2004) or (contrariwise) very narrowly (Steyaert 1972, 1980). Bazzalo & Wright (1982) and other authors, especially when synonymizing various different species under the same name, considered context color of little value. On the other hand, Steyaert (1967a) regarded the resin-like deposits as unimportant, mainly in the species of subg. *Elfvingia*. However, we found that these deposits together with context color are important in separating taxa. The resin-like deposit (termed "melanoid deposits" by Gottlieb & Wright, 1999 and melanoid substance by Steyaert, 1980) was called by Ryvarden (2000) "resinous bands," the term adopted here but more narrowly so as to differentiate actual resinous bands from more generalized resinous incrustations (small, fragmented deposits that do not form bands).

Ganoderma basidiospores have been traditionally described as double walled with a thin exosporium and thick endosporium, echinulate or with inter-walled pillars, or struts, or sinuous ridges, with truncate apex (Patouillard 1889, Murrill 1902, Haddow 1931, Overholts 1953, Corner 1983, Tham 1998, Ryvarden 2000, 2004 among others). The terms exosporium, episporium, and endosporium have been differently applied for the authors mentioned above if Clémençon (2004) is followed. Steyaert (1977) described the basidiospores with epi-endosporium and echinulate ecto-perisporium, sometimes the ecto-perisporium crestate besides echinulate. Bazzalo & Wright (1982), who used "perisporium" and "endosporium," considered the ornamentation as derived from the endosporium, in contrast to Clémençon (2004), who determined that the exosporium forms the ornamentation. As defined by Kirk et al. (2008), echinulate (diminutive of echinate: "having sharply pointed spines") does not apply to the inner ornamentation seen in *Ganoderma,* so we prefer to describe it as pillars. Bazzalo & Wright (1982) and Gottlieb & Wright (1999),



Figs. 26–34. *Ganoderma* crustohymeniderm cells (26–31): **26**. Cylindrical cells with granulations (arrow) of *G. perzonatum*. **27**. Cylindrical cells with incrustation (arrow) of *G. stipitatum*. **28**. Clavi-form cells without granulations of *G. sessiliforme*. **29**. Irregular cell with apical branches and protuberance of *G. multiplicatum*. **30**. Lobulate-branched cells with many protuberances of *G. oerstedii*. **31**. Lobulate-branched cell with a large lateral branch and a smaller apical branch of *G. multiplicatum*. **32–34**. Hymenial cystidia (32. *G. sequoiae*; 33. *G. perzonatum*; 34. *G. stipitatum*).

who observed the basidiospore pillars under SEM, proposed them as useful in determining species. We emphasize their usefulness by noting that not only are the pillars seen through SEM, pillar characters like size and disposition are also visible through light microscopy.

The basidiospore cap has been variously named: "bouchon perisporique" (Heim 1962), apical papilla (Furtado 1962), umbo (Corner 1983), and cap (Tham 1998). As mentioned above, when the cap collapses and breaks the basidiospore apex looks truncate; in few cases appears subacute, because the cap is very small or even absent. The term subacute, described by Steyaert (1972) for basidiospores with unchanged apex or apex unaltered at maturity, was adopted by Corner (1983), although this character has not been used systematically. We consider that it is an important feature in diagnosing taxa; for example, it supports *G. perturbatum* as independent from *G. resinaceum* and related to *G. dorsale*. *Ganoderma concinnum*, *G. dorsale*, *G. longistipitatum*, and *G. perturbatum* form a distinctive group of species by their stipitate basidiomata and subacute basidiospore apices.

Basidiospore shape is also an important diagnostic character. Oblong to cylindrical spores differentiate *G. ravenelii* and *G. zonatum* from morphologically similar species such as the ellipsoid spored *G. curtisii* and *G. orbiforme.*

Pileus surface is a complex structure in which the crustohymeniderm cells, hyphae and resinous-like substances are immersed. Many discussions on its taxonomic importance in *Ganoderma* have been made (e.g. Furtado 1965a, Gottlieb & Wright 1999, Steyaert 1980); however, its current use is limited, perhaps caused by a lack in precise terminology. Much of the complexity is caused by the great variability in the branching pattern of the cells in the same basidioma, creating confusion and difficulty to find a collective term. Torres-Torres & Guzmán-Dávalos (2005) realized that pileipellis cells vary greatly in size and shape depending on the site sampled and on the basidiome age. For this reason, tissues should be cut away from the basidiome edge, and only mature cells must be compared.

Imazeki (1939) and Steyaert (1980) classified genera based on pileipellis (dermis) type. Bazzalo & Wright (1982) had regarded pileipellis types described by preceding authors as valuable for identifying large groups but not useful for delimiting species. Subsequently, Gottlieb & Wright (1999) considered three general dermis types based partly on Steyaert (1980): a) "hymeniodermis *vera,*" b) "hymeniodermis formed by diverticulate hyphae… with similar endings, though wider through the apex, and with lateral branches, acanthophyses-like," and c) "hymeniodermis spheroid-pedunculate… composed of claviform filaments, with or without lateral diverticules, but always with emerging spheroid-pedunculate or capitate knobs at the apex." Their acceptance of these dermis types and basidiospore ornamentation as useful for delimiting taxa has

been confirmed in the present investigation. However, we found it difficult to assign species to one of these pileipellis types, we prefer to describe cells according to shape and the number of branches and protuberances. There is one species complex — *G. boninense*, *G. multiplicatum*, *G. orbiforme*, *G. subfornicatum* and *G. oerstedii*, all with yellowish-brown to reddish-brown context and pileipellis cells with protuberances and/or branches — in which species are differentiated base on basidiospore features and pileipellis cell shape and branch numbers.

Karsten (1881) did not mention cystidia in his original description of *Ganoderma*, and many mycologists accepted his non-cystidiate concept (Haddow 1931, Heim 1962, Furtado 1965b, 1967, Steyaert 1967b, 1972, Bazzalo & Wright 1982, Gilbertson & Ryvarden 1986, Ryvarden 1991, 2000). Although Corner (1983) noted that Demelius (1919) referred to acute 'cystidioles' in *G. applanatum* (Pers.) Pat. (subg. *Elfvingia*) and *G. lucidum* (Curtis) P. Karst., we follow Vellinga (1998) and regard them as true cystidia that are present in at least five species.

In conclusion, we consider that additional important features for characterizing *Ganoderma* species that can be directly observed or under light microscopy should include context color, structure, and resinous deposits; basidiospore pillar disposition and size; and pileipellis cell shape, protuberances, branches, and granulations.

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Four new polypore species from the western United States

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Abstract — Four new species of polypores are described from California, Oregon, and Washington, U.S.A. *Antrodia madronae,* seemingly confined to dead stems and branches of *Arbutus menziesii,* is closely related to European *Antrodia sandaliae* growing on *Arbutus unedo*. The generic position is evaluated for *Ceriporiopsis pseudoplacenta,* a strikingly colored species with many unique features that seems surprisingly common in the northwestern states. *Fuscoporia palomari* is distinguished from the similar *F. viticola* by much larger pores and spores. *Skeletocutis subodora* differs from *S. odora* by having thick subiculum, differently shaped basidiospores, abundant cystidioles, and no skeletal hyphae in the context. The nrDNA ITS region of all new species gave unique sequences in GenBank.

Key words — *Hymenochaetaceae, Phellinus, Phlebia,* ribosomal DNA, taxonomy

Introduction

The forested west coast of the United States is extremely biodiverse due to its varied topography, climate zones, and geology. The number of vascular plant species found in California exceeds the total number from the central and northeastern United States and adjacent Canada, an area ten times larger (CI 2007). The high degree of plant endemism is also striking; more than 61% of Californian plant species are found nowhere else in the world. This creates favorable conditions also for high diversity and endemism of wood-bound poroid fungi. Nevertheless, in the North American polypore monograph by Gilbertson & Ryvarden (1986, 1987), only 60 (of 406) species are specified as occurring in the western states of California, Oregon, and Washington. This indicates that the region still has a great potential for field research in polyporology.

Few substantial contributions have been published on USA west coast polypores since Murrill's early papers (Murrill 1912, 1915). Overholts, who

described several new species sent to him from the Pacific coast states, never collected in the far west (Overholts 1939). The same is essentially true for Lowe and other distinguished USA polyporologists. Gilbertson (1976, 1979), who published some new species and important distribution data, focused his research primarily on the dry hot regions of Arizona.

We visited various localities on the USA west coast and report here on several polypore collections. Although the collected material was quite scarce, we describe four new species supported by distinctive macro- and microscopic features and unique ITS rRNA sequences.

Materials & methods

Polypores were collected in August (2001, 2003), September (2005, 2009), and April (2010) during one-week trips to various localities in California, Oregon, and Washington (Vlasák 2008). The specimens were dried and microscopically inspected in Melzer's reagent (IKI) and 5 % KOH. Similar fungi from other parts of USA and Europe were also collected or loaned from herbaria and studied in detail as noted in 'Specimens studied.' All collected specimens are deposited in private herbarium of the first author, with types and some paratypes also in PRM. DNA isolation from critical samples, nr DNA ITS region sequencing, and comparative phylogenetic analyses methods follow Vlasák & Kout (2011). The evolutionary history was inferred using the Neighbor-Joining method (Saitou & Nei 1987) and phylogenetic analyses were conducted in MEGA4 (Tamura et al. 2007).

Taxonomy

Fuscoporia palomari Vlasák & Ryvarden, **sp. nov.** PLATES 1–2

MYCOBANK MB 563062

Basidioma resupinatus vel effuso-reflexus, pori facies brunnea, rotundi vel elongati, 2 pro mm. Setae subulatae, brunnei, 40–75 × 5–8 µm, basidiosporae hyalinae, leves, cylindricae, 8–11 × 2.7–3.5 µm, inamyloideae, indextrinoideae.

TYPE: USA. California: Palomar Mt. State Park, 1500 m above see level, on oak log, 16 Apr 2010, J. Vlasák Jr. JV1004/5-J (**Holotype**, PRM 915982; **isotype**, JV1004/5a-J, GenBank JN592493).

ETYMOLOGY: Referring to the type locality on Mt. Palomar, CA.

BASIDIOME about 7 × 5 cm, resupinate to effused-reflexed, with sharply delimited sterile margin 0.5–1 mm wide, pilei indistinct, projecting only 2–3 mm and ≤ 1 cm wide, upper surface tomentose, pore surface yellowish brown with a shiny luster when viewed obliquely. PORES large, circular to angular, in places somewhat labyrinthine, 2 per mm, with thin, soft dissepiments that are, though, often thickened on pore edges. CONTEXT brown, fibrous, rather soft, very thin in resupinate part but up to 2 mm thick in developing pilei; tube layer concolorous and continuous with the context, up to 3 mm thick. CONTEXTUAL HYPHAE of two types, some brown in KOH, thick-walled, 2–4 µm in diam.,

Plate 1: *Fuscoporia palomari*. Basidiome.

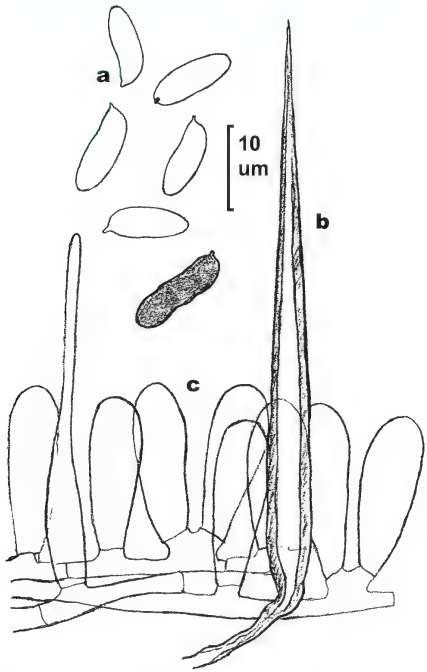

Plate 2: *Fuscoporia palomari* microscopic structures.
a: Basidiospores, b: Hymenial seta, c: Hymenium with basidia and cystidiole.

others hyaline in KOH, thin-walled, with occasional branching, simple-septate, 2–3 µm in diam.; tramal hyphae similar. Setae abundant, narrowly subulate,

thick-walled, very long, 40–75 × 5–8 µm, most of them around the upper length limit, protruding perpendicularly from the hymenium to more than a half of their length. Basidia clavate, simple-septate at the base, 15–25 × 5–8 µm. Cystidioles hyphoid, rarely present, with up to 25 µm long and about 1.5 µm broad terminal part. Basidiospores cylindric, straight, hyaline, thin-walled, smooth, IKI–, 8–10(–11) × 2.7–3.5 µm, few turning brown in KOH. Causing a white rot.

Comments — Only one juvenile basidiome with rudimentary developing pilei was collected. However, it shows many unique features: very large, hyaline cylindric spores, long setae, and large, shining pores. The hyaline basidiospores do not change in KOH except for a small fraction where the inner contents (not walls) turn deep brown. These usually have rather uneven walls and distorted shapes (Plate 2) that we consider atypical. The ITS nrRNA sequence is unique, showing significant similarity (max 93%) in GenBank Blast search only with *Fuscoporia viticola* (Schwein.) Murrill, a species with similar long setae and cylindric (but much narrower) basidiospores. *Fuscoporia sentifera* (T. Hatt.) Y.C. Dai, originally described from Japan (Hattori 1999) and recently reported in China (Dai 2010), is similar to *F. palomari* by resupinate to effused-reflexed basidiocarps, long hymenial setae, and cylindric spores but differs by a hispid pileal surface and smaller spores (5.8–7 × 2–2.5 µm).

Antrodia madronae Vlasák & Ryvarden, **sp. nov.** Plates 3–4

MycoBank MB 563081

Basidiomata annua, resupinata, nodulosa vel aliquando pileata, colore ligneo-pallens, pori rotundi vel elongati et sinuosi, 2–3 pro mm. Basidiosporae hyalinae, leves, tenuitunicatae, cylindraceae, 10–12 × 2.8–3.8 µm, inamyloideae, indextrinoideae. Cariem brunneam in ligno Arbuti menziesie *producet.*

Type: USA. Oregon: Oregon Caves entrance, on dead branch of pacific madrone tree, 14 Sep 2007, J. Vlasák Jr. JV0709/117-J (**Holotype**, PRM 899296; GenBank JN592494).

Etymology: Referring to the only known substrate tree, pacific madrone, *Arbutus menziesii* Purrs (*Ericaceae*).

Basidiomata annual, up to 30 × 10 cm, resupinate to effused-reflexed, nodulose or with narrow sloping pilei developed on a decurrent pore surface, individual pilei up to 4 cm wide, soft when fresh, upper surface ochraceous to light brown colored, coarsely rugose, azonate, margin sharp, tomentose, pore surface concolorous. Pores circular when immature, (1–)2(–3) per mm, on sloping parts often sinuous and elongated; dissepiments thick. Context thin and about the same color as the pore surface, tube layer concolorous, up to 7 mm thick. Hyphal system dimitic, generative hyphae clamped, branched, thin-walled or somewhat thick-walled, 2–4 mm in diam., dominating in all parts of basidiocarp, thickened walls gelatinizing and dissolving in KOH,

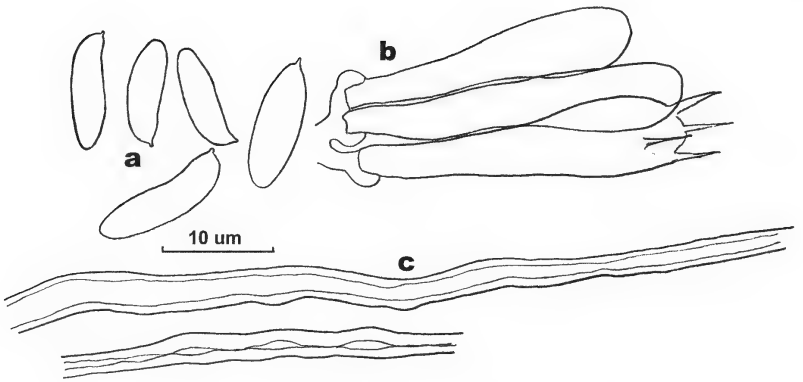

Plate 3: *Antrodia madronae* microscopic structures.
a: Basidiospores, b: Basidia, c: Skeletal hyphae.

Plate 4: *Antrodia madronae*. Basidiome (JV0709/90).

skeletal hyphae thick-walled but never solid, 3–5 µm in diam., dissolving in KOH. Cystidia none. Basidia narrowly clavate, with four sterigmata and a basal clamp, 25–40 × 6–8 µm. Basidiospores hyaline, thin-walled, smooth, IKI–, cylindrical or somewhat fusiform, 10–12 × 2.8–3.8 µm. Causing a brown rot on dead branches and stems of pacific madrone.

Additional specimens examined: ***Antrodia madronae*** – **USA. California:** Crescent City, Myrtle Creek, on dead stem of *Arbutus menziesii,* 12 Sep 2007, J. Vlasák (JV0709/90). ***Antrodia sandaliae*** – **ITALY. Sardinia:** Montarbu Forest, Sarolegne,

Arbutus unedo, 29 Nov 2003, A. Bernicchia (HUBO 7803, GenBank JN592495); 29 Nov 2003, A. Bernicchia (HUBO 7784, ITS sequence is identical with that of HUBO 7803); 24 Nov 1999, A. Bernicchia (HUBO 7340).

Comments — More material is needed to describe the extent of macroscopic variation of this species. The description is based on only two specimens; the type is a small, resupinate basidiocarp in the wood-crevice of a dead, broken branch of a living tree, while the other, growing on a dead standing tree, is a large, partly pileate basidiocarp with a strikingly rugose pileus surface but old and heavily damaged by insects (Plate 4). *Antrodia madronae* is characterized by large cylindrical basidiospores, rather soft fruitbodies dominated by generative hyphae, extremely soft trama that almost dissolved during the microscopic preparation in Melzer's reagent, large pores, and causing a brown rot. Undoubtedly, the fungus is closely related to the Mediterranean *A. sandaliae* Bernicchia & Ryvarden, also growing on an arbutus (*Arbutus unedo* L.) and characterized by a white pulvinate basidiome, similar soft trama and hyphal system, and similarly shaped but smaller basidiospores (7.5–10.0 × 1.8–3.0 μm; Bernicchia & Ryvarden 2001, Gorjón & Bernicchia 2009). Although both species have KOH-soluble thick-walled hyphae, the pores, basidia and spores of *A. madronae* are distinctly larger and the basidiome is varicolored.

Sequence analysis — The amplified ITS1-5.8S-ITS2 rDNA region of *A. madronae* was 565b long between ITS1 and ITS4 primer sequences (White et al. 1990) and the sequence was rather similar to that of *A. sandaliae* HUBO 7803 and HUBO 7784. The sequence variation between *A. madronae* and *A. sandaliae* of 2.0% (4/199) in ITS1 and 2.4% (5/212) in ITS2 suggests a very close relationship regardless of the pronounced macroscopical differences. Other species in GenBank Blast Search are only distantly related, with *A. albobrunnea* (Romell) Ryvarden (max. 83% sequence similarity) seemingly the closest.

Ceriporiopsis pseudoplacenta Vlasák & Ryvarden, **sp. nov.** Plates 5–8

MycoBank MB 563082

Basidiomata annua, resupinata, salmon pink pallide colore sed carnosa et cartilaginea post siccatione, deinde rubro-brunnei colore. Pori rotundi vel angulati, 3–4 pro mm. Systema hypharum monomiticum, hyphae hyalinae, fibulatae, ramosae, 2.0–3.0 μm latae, quae materiam oleaceam continent. Basidiosporae hyalinae, leves, ellipsoideae, 3.5–4.5(–5) × 2.2–3 μm.

Type: USA. Washington: Forks, Bogachiel State Park, 6 Aug 2003, *Picea sitchensis* (Bong.) Carrière. log, J. Vlasák JV0308/68 (**Holotype**, PRM 899297; **isotype**, JV0308/68a; GenBank JN592496, JN592497, JN592504).

Etymology: from the Latin for false placenta, based on the superficial similarity with *Postia placenta*.

Basidiomata annual, resupinate, up to 20 × 10 cm, soft when fresh, tough and fleshy when dry, and soaked with resinous substance, not readily separable;

Plate 5: *Ceriporiopsis pseudoplacenta*. Basidiome in situ (JV0709/54).

pore surface pale apricot to salmon-pink when fresh, exuding white drops, turning up dark reddish brown on drying. Pores circular to angular, 3–4 per mm, with thick, entire dissepiments that finally become thin and lacerate; margin narrowly sterile but sometimes up to 15 mm broad in actively growing stage, whitish at first, later pale apricot, on drying dark reddish brown to black and often peeling off. Context pale orange-pinkish when fresh, blackish when dry, less than 1 mm thick; tube layer pale salmon-pink, up to 5 mm thick. Hyphal system monomitic, hyphae hyaline, thin-walled, often branched, 2–4 µm in diam., with abundant, often closely spaced clamps. In Melzer's reagent, the hyphae are covered by small oil drops that often fuse into large, brownish, oily blotches; in KOH the hyphae are stripped of the oily matter that forms irregular, brown aggregates in the tissue. Cystidia none. Basidia inconspicuous, not much broader than hyphal ends, thin-walled, narrowly clavate, 4 -sterigmate, with a basal clamp, 15–25 × 4–5 µm,. Basidiospores broadly ellipsoid, distinctly narrowing to the apex in most cases, hyaline, thin-walled, smooth, IKI–, 3.5–4.5(–5) × 2.2–3 µm. On rather rotten logs of various coniferous trees. White rot.

Additional specimens examined: ***Ceriporiopsis pseudoplacenta*** – **USA. Washington**: Hoh River, conifer log, 6 Aug 2003, J. Vlasák (JV0308/61, PRM 899298, ITS sequence identical with JN592497); **California**: Kings Canyon Nat. Park, 12

Plate 6: *Ceriporiopsis pseudoplacenta*. Basidiome in situ (JV0709/53).

Aug 2001, conifer log, J. Vlasák (JV0108/82A, PRM 899299); Crescent City, Jed Smith State Park, Simpson Grove, hemlock, 8 Sep 2007, J. Vlasák (JV0709/53, PRM 899300, GenBank JN592498, JN592505); 8 Sep 2007, J. Vlasák (JV0709/54); **Tennessee**: Great Smoky Mt., Gregory Ridge Trail, hemlock, Sep 2005, J. Vlasák (JV0509/52, GenBank JN592499, JN592506). ***Auriporia aurea*** – **USA**. California: Sequoia Nat. Park, Wolverton Area, *Abies magnifica* A. Murray log, 14 Sep 2001, J. Vlasák (JV0109/92, PRM 915966, GenBank GU595152). ***Auriporia aurulenta*** – **CZECH REPUBLIC**. Red Marsh Nat. Preserve, *Pinus sylvestris* L., 25 Oct 2009, J. Vlasák (JV0910/12, PRM 915967, GenBank GU595153). ***Ceriporiopsis* sp.** – **USA. Florida**: Everglades Nat. Park, Long Pine Key, 20 Apr 2009, *Pinus elliottii* Engelm., J. Vlasák (JV0904/46, GenBank JN592500, JN592507). ***Postia placenta*** – **USA. New York**: Upper Jay, Adirondacks Park, *Pinus* sp., 20 Sep 2005, J. Vlasák (JV0509/174, GenBank JN592502); **California**: Yosemite Nat. Park, Tuolumne Grove, *Pinus lambertiana* Douglas, 17 Aug 2001, J. Vlasák (JV0108/98, GenBank JN592501). **SLOVAK REPUBLIC**: Dobroc Virgin Forest, 21 Sep 2009, *Picea abies* L. (H.Karst.), J. Vlasák (JV0909/16, GenBank JN592503).

Comments — *Ceriporiopsis pseudoplacenta* is probably widely distributed in the western USA, as we found it in various locations during every trip to California and Washington (Vlasák 2008). One specimen comes also from the Great Smoky Mt., Tennessee. It is difficult to understand why this strikingly colored fungus has not previously been described, yet we found nothing similar in the literature. In the field, *C. pseudoplacenta* can be mistaken for *Auriporia aurea* (Peck) Ryvarden or *Postia placenta* (Fr.) M.J. Larsen & Lombard because

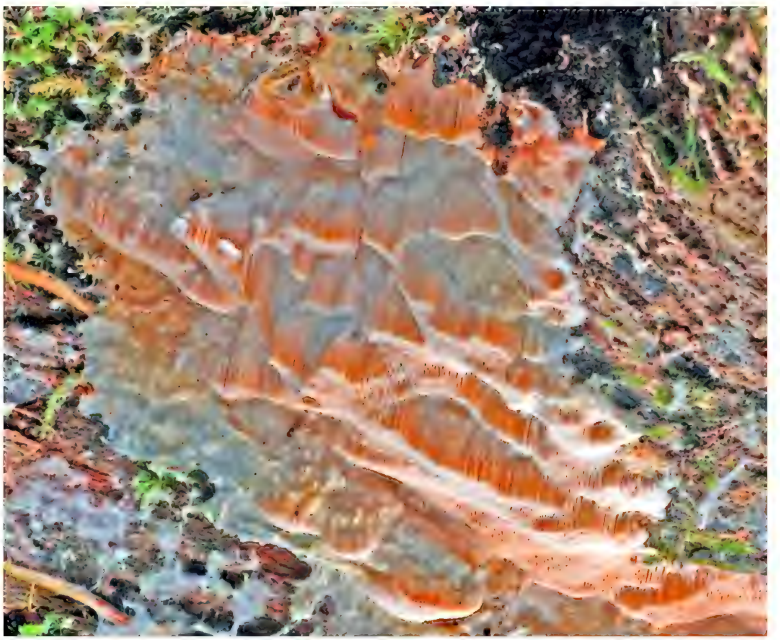

PLATE 7: *Ceriporiopsis pseudoplacenta*. Basidiome in situ (JV0509/52).

of similar colors and soft, fleshy consistency. *Auriporia aurea* (and the similar European *A. aurulenta* A. David, Tortič & Jelić) differ by having skeletal hyphae and thick-walled cystidia. *Postia placenta* is difficult to separate by microscopy alone because both species have similar hyphal system with no special structures and similar spores that are, however, smaller in *C. pseudoplacenta.* Macroscopical differences are more distinctive, especially when monitoring basidiome changes during development and drying. *Postia placenta* starts growing usually as a deep-red basidiome, changing to rosy-pink and, when old or dry, to whitish-pink or light ochre. *C. pseudoplacenta* follows just the opposite coloration steps; it starts as whitish or light salmon pink, becoming increasingly more deeply red until maturing to red brownish or black in the subiculum and margin. Also, the consistency of dried fruitbodies is quite different: soft and brittle in *P. placenta* but tough and rubber-like (reminiscent of beef jerky) in *C. pseudoplacenta*. *Auriporia aurea, A. aurulenta,* and *P. placenta* cause a dry brown rot. The wood under *C. pseudoplacenta* basidiomes is usually also quite dark as the fungus causes some red-brownish wood coloration, but its stringy fibrous structure with small pockets corresponds more to white rot. Unfortunately, we did not perform oxidase tests on fresh fruitbodies. *Ceriporiopsis gilvescens* (Bres.) Domański, a white rot polypore, also sometimes resembles *C. pseudoplacenta,* but its salmon-pinkish colours are usually

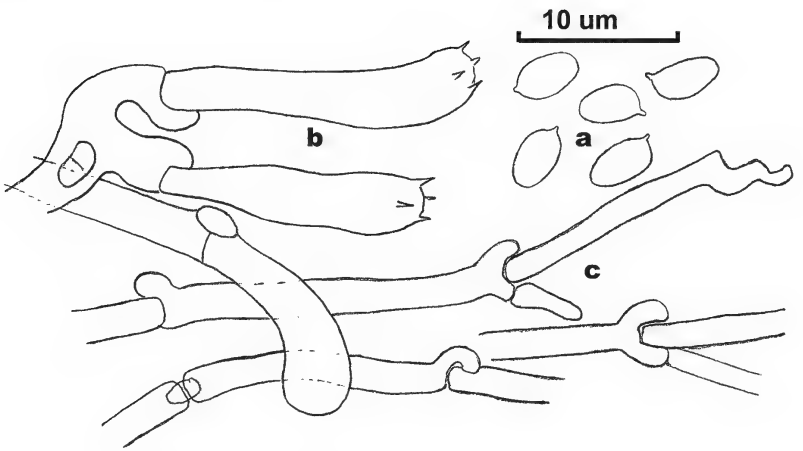

Plate 8: *Ceriporiopsis pseudoplacenta* microscopic structures.
a: Basidiospores, b: Basidia, c: Hyphae.

paler and the spores are narrower (1.5 – 2 µm). *Antrodiella albocinnamomea* Y.C. Dai & Niemelä, widely distributed in Asia (Dai & Niemelä 1997; Dai 2012), is macroscopically similar to *C. pseudoplacenta*, but differs in its dimitic hyphal structure and oblong-ellipsoid spores (3.7–5 × 2.1–2.9 µm).

The taxonomic position of *C. pseudoplacenta* is a mystery, as the sequence analysis (see below) revealed very low homology with known fungi. So we chose for it the congregate genus *Ceriporiopsis* Domański that encompasses white rot species that are poroid, resupinate, and monomitic with clamp connections.

Sequence analysis — Successful amplification and sequencing of the *C. pseudoplacenta* ITS (nrDNA) region from four of six collected specimens showed only minor differences between individual specimens (Plate 9). 5.8S rDNA is highly homologous with many other polypores, but for the very long ITS1 region (280-283b) the GenBank MegaBlast search found "no significant similarity," which we never experienced before for polypore species. ITS2 analyses indicate very low similarity with seemingly distant fungi, with *Ceriporiopsis* species showing the closest support among the polypores. Sequence analyses weakly support clustering of *C. gilvescens* with *C. pseudoplacenta,* and apart from the brown-rot fungi *Postia* Fr., *Auriporia* Ryvarden and *Antrodia* P. Karst. in a separate clade (Plate 9). Some *Phlebia* Fr. species, macroscopically similar except for the hymenophore form, cluster even more closely with *C. pseudoplacenta*, as also shown in the nrDNA LSU sequence analyses (Plate 10). For the time being, the generic position of the new species must be regarded as provisional.

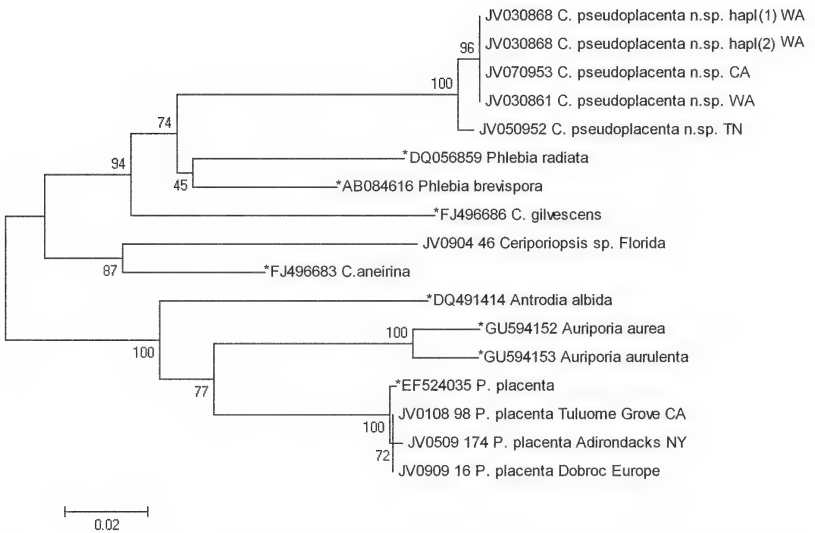

Plate 9. Evolutionary relationships using the Neighbor-Joining method of 16 *Antrodia*, *Auriporia*, *Ceriporiopsis*, *Phlebia,* and *Postia* specimens based on nr DNA ITS region sequence. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances are in the units of the number of base substitutions per site. Accession numbers with * represent sequences retrieved from GenBank. All other GenBank accession numbers refer to new sequences listed in this paper.

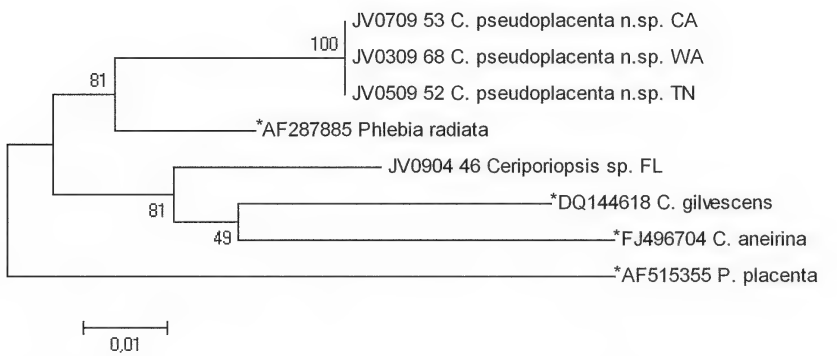

Plate 10. Evolutionary relationships of 8 *Ceriporiopsis*, *Phlebia* and *Postia* specimens based on nrDNA LSU region sequence. Phylogenetic analysis as referred in pl. 9.

Another *Postia placenta*-like specimen (JV0904/46, Plate 9) we have collected in early spring from the tropical region of southern Florida from slough pine (*Pinus elliottii*) is morphologically very similar to *P. placenta* or *C. pseudoplacenta* except for slightly shorter spores, but its sequence is more similar to *Ceriporiopsis aneirina* (Sommerf.) Domański. We need additional material before resolving the identity of this specimen.

Skeletocutis subodora Vlasák & Ryvarden, **sp. nov.** Plates 11–13

MycoBank MB 563083

Basidiomata annua, resupinata, usque ad 10 cm lata et 1 cm spissa, colore albicante, pori rotundi vel angulati, 3–5 pro mm, contextus albus, 1–4 mm crassus. Systema hypharum monomiticum, hyphae fibulatae, cystidioli hyalinni, fusiformes, basidiosporaes hyalinae, leves, tenuitunicatae, non amyloideae, non dextrinoideae, 5–6 × 2.0–2.3 µm.

Type: USA. Oregon: Crater Lake visitors center, *Pseudotsuga menziesii* (Mirb.) Franco log, 15 Sep 2007, J. Vlasák JV0709/134 (**Holotype**, PRM 899301; **isotype**, JV0709/134a, GenBank JN592509).

Etymology: From the Latin for *odora*-like, referring to the similarity to *S. odora*.

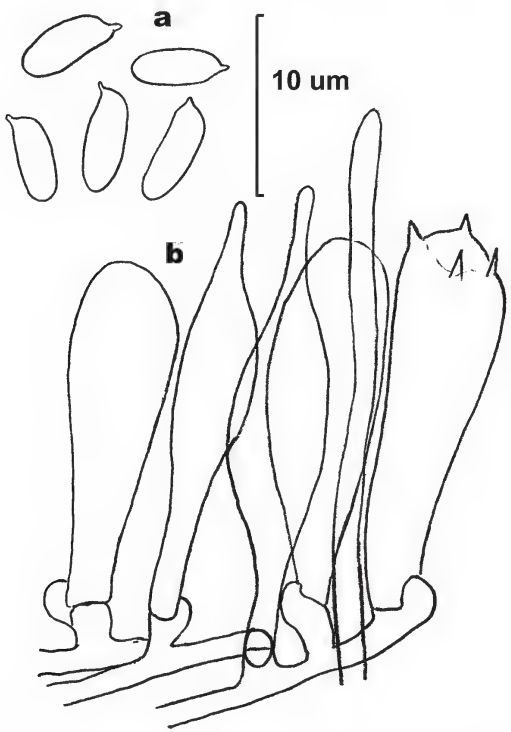

Plate 11. *Skeletocutis subodora* microscopic structures.
a: Basidiospores, b: Hymenium with basidia and cystidioles.

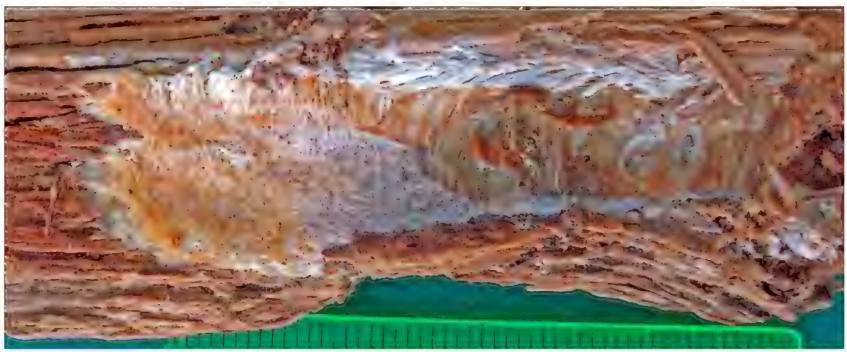

PLATE 12. *Skeletocutis subodora*. Basidiome (PRM 899301, holotype)

BASIDIOMATA annual, resupinate, found in two patches effused to 5 × 5 and 10 × 10 cm, up to 1 cm thick, soft when fresh, no odor noted, drying brittle and shrinking; sterile margin 1–3 wide, well demarcated, whitish, tawny on drying; pore surface cream-colored, drying pale beige. PORES at first regular, 3–5 per mm, with very thin dissepiments, on drying becoming more dented and more irregular. SUBICULUM white to pale cream, 1–4 mm thick; tube layer concolorous with pore surface, resinous and compressed when dry, distinctly ochre-colored compared with the white subiculum, up to 8 mm thick. HYPHAL SYSTEM monomitic, hyphae hyaline, thick to thin-walled, often branched, with clamps, 2–4 µm in diam., their ends on dissepiments edges sparingly incrusted by sharp crystals. CYSTIDIAL ELEMENTS as fusoid cystidioles abundant and conspicuous, thin-walled, with a basal clamp, 15–25 × 2–6 µm. BASIDIA clavate, 4-sterigmate, with a basal clamp, 15–20 × 5–6 µm. BASIDIOSPORES narrowly ellipsoid to cylindrical, hyaline, thin-walled, smooth, IKI–, 5–6 × 2–2.3 µm. On decorticated log of Douglas fir, causing a white pocket rot.

ADDITIONAL SPECIMENS EXAMINED: ***Ceriporiopsis guidella*** – **ITALY**: Ris. Di Sasso Frantino, Rio Cullacce (Forli), *Acer* sp., 22 Sep 2001, A-R. Bernicchia (coll. 7731). ***Skeletocutis diluta*** – **USA. FLORIDA**: Everglades Nat. Park, Long Pine Key, *Pinus elliottii*, 29 Aug 2010, J. Vlasák (collection JV1008/61, PRM 899302, GenBank JF692198). ***Skeletocutis odora*** – **CZECH REPUBLIC**. Zofin Nat. Preserve, *Picea abies* log, 22 Jul 2010, J. Vlasák (JV1007/7, GenBank JN592509).

COMMENTS. The fungus was collected as a probable *Skeletocutis odora* (Sacc.) Ginns, which is actually very similar macroscopically and with a sequence that shows a high degree of homology (see below). The most important macroscopical diagnostic feature is the thick (≤ 2–4 mm) subiculum, barely 1 mm thick in *S. odora*. The distinctive (relatively broad non-allantoid) basidiospores, conspicuous large cystidioles, and monomitic context also distinguish *S. subodora*. The macroscopically similar *Ceriporiopsis guidella* Bernicchia & Ryvarden (Bernicchia & Ryvarden 2003) also produces similar

cystidioles and spores but differs in an extremely thin subiculum, growth on hardwoods, and different ITS nrRNA sequence (see below).

Thus far the new species is known only from the type locality, although the occurrence of the similar *Skeletocutis odora* in the USA warrants mention. Lowe (1966) stated that *S. odora* is known with certainty only from Peck's type and one other report from Pennsylvania, while Gilbertson & Ryvarden (1987) do not cite the species at all from the USA. We also never found *S. odora* during numerous excursions in either the east or west USA, although it is not uncommon in old-growth conifer forests in Europe.

Sequence analysis — The amplified ITS1-5.8S-ITS2 sequence from *Skeletocutis subodora* (603b long between ITS1 and ITS4 primers) was somewhat similar (variations of 3.0% (6/195) for ITS1 and 4% (8/205) for ITS2) to that of *S. odora* (Plate 13), suggesting that the two species are related to but different from each other. Surprisingly, *Tyromyces chioneus* (Fr.) P. Karst., with a still unsettled taxonomic position, shows surprisingly more homology than other *Skeletocutis* species.

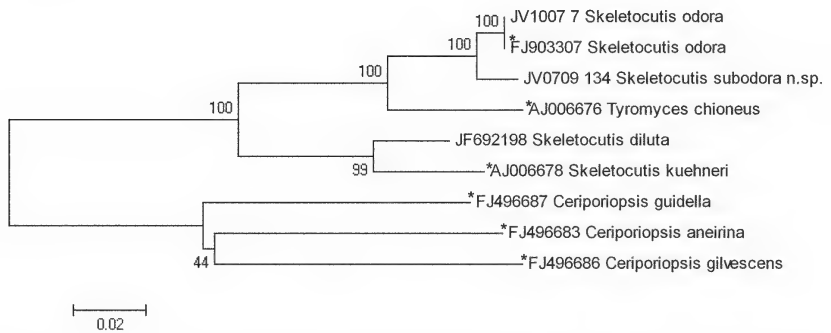

Plate 13. Evolutionary relationships of 9 *Skeletocutis* and *Ceriporiopsis* specimens based on nr DNA ITS region sequence. Phylogenetic analysis as referred in pl. 9.

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Studies on Croatian *Basidiomycota* 2: *Marasmiellus milicae* sp. nov.

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Abstract — A new species, *Marasmiellus milicae,* is described from the Mediterranean part of Croatia and compared with similar species. It is characterized by having rather small, white pileus, distinctly to strongly eccentric, curved, short stipe, white hymenophore becoming dark brown when dry, (sub)amygdaloid basidiospores, mostly diverticulate cheilocystidia, pileipellis and stipitipellis with strongly developed Rameales-structure, and gelatinized trama. Color photographs and line drawings of microscopic elements and basidiomata accompany the description.

Key words — biodiversity, mycobiota, *Omphalotaceae,* taxonomy

Introduction

During the last decade of intensive research of Croatian *Basidiomycota,* a significant number of rare and new species has been found. Some of these have been published in the last few years (Hausknecht et al. 2007; Mešić & Tkalčec 2008, 2009; Tkalčec & Mešić 2008; Tkalčec et al. 2009) and the remaining will be published in a series of papers under the title "Studies on Croatian *Basidiomycota,*" of which this is the second. In the present paper we describe a new species of *Marasmiellus* (*Omphalotaceae*, *Agaricales*) found in the Mediterranean part of Croatia.

Materials & methods

The description of *Marasmiellus milicae* is based on two collections consisting of 27 basidiomata in total. The macroscopic description is based on fresh basidiomata. Microscopic features are described from dried material mounted in potassium hydroxide (KOH) solution and Melzer's reagent, and observed with a light microscope (brightfield and phase contrast – PhC) under magnification up to 1500×. Spore measurements do not include the apiculus. Arithmetic means of spores length and width are shown in italic font in the center of the measurements. The length/width ratio of all measured spores is given as the "Q" value (min. – av. – max.), and the range of arithmetic averages

of "Q" value (Q_{av}) of both collections is also given. The holotype and additional collection are deposited in the Croatian National Fungarium, Zagreb, Croatia (CNF), while an isotype is deposited in the Herbarium of the Moravian Museum, Brno, Czech Republic (BRNM).

Taxonomy

Marasmiellus milicae Mešić, Tkalčec & Antonín, **sp. nov.** FIGS 1–10

MycoBank MB 561574

Differs from the most similar species by its white pileus; short, eccentric, dark stipe; white hymenophore becoming dark brown when dry; (sub)amygdaloid spores on average 9.1 × 5.4 µm; mostly diverticulate cheilocystidia; and strongly developed pileipellis and stipitipellis Rameales-structure.

TYPE: Croatia, island of Mljet (near Dubrovnik), near Prožurska Luka village, 42°43′51″N 17°38′44″E, alt. 60 m, 9 November 2009, leg. M. Čerkez & Z. Tkalčec (**Holotype** CNF 1/5692, **Isotype** BRNM 736106).

ETYMOLOGY: Named after Croatian mycologist Dr. Milica Tortić (1920–2008).

PILEUS 6–18 mm broad, hemispherical to convex, margin involute at first, then inflexed to deflexed, slightly crenulate when young, surface smooth to entirely rugose, finely tomentose, white, sometimes with grayish to greenish-gray tinge. LAMELLAE adnate, distant, L = 8–12, l = 0–2(–3), strongly intervenose, especially in larger specimens, sometimes furcate, often ± sinuous, white with concolorous edge, whole hymenophore becoming dark brown when dry. STIPE 1–3 × 0.5–1.2 mm, distinctly to strongly eccentric, curved in horizontal direction (basidiomata laterally attached to the substratum), cylindrical with slightly broadened apex, insititious, pubescent, white when young, then becoming dark gray to gray-black, sometimes with greenish tinge at the apex. CONTEXT concolorous with outer surface. SMELL very weak, fungoid when cut.

BASIDIOSPORES 8–*9.1*–10 × 4.5–*5.4*–6.0 µm, average = 9.0–9.3 × 5.3–5.7 µm, Q = 1.5–*1.70*–1.98, Q_{av} = 1.63–1.73, mostly (sub)amygdaloid in side view, ellipsoid to fusoid in frontal view, thin-walled, hyaline, apiculus ca. 1.0 × 1.3 µm. BASIDIA 28–47 × 6.0–10 µm, 4-spored, clavate. BASIDIOLES 15–45 × 3.0–10 µm, clavate, cylindrical, subfusoid. LAMELLAR EDGE heterogeneous. CHEILOCYSTIDIA (10–)13–60 × 5.0–14 µm, clavate, subcapitate, (sub)cylindrical, fusoid, mostly with simple, branched to coralloid apical projections (digitate, conical, or obtuse) up to 30 × 5.0 µm; thin-walled, hyaline. PLEUROCYSTIDIA absent. PILEAL AND HYMENOPHORAL TRAMA gelatinized, hyphae cylindrical, thin-walled, sometimes branched, up to 10 µm wide. PILEIPELLIS an ixocutis composed of ± cylindrical, thin- to slightly thick-walled, smooth or minutely incrusted, diverticulate or smooth, up to 7.0 µm wide hyphae; terminal cells and lateral projections up to ca. 20 × 3.0 µm, digitate, branched to coralloid, obtuse (well-developed Rameales-structure). STIPITIPELLIS a cutis composed

Figs 1–3. *Marasmiellus milicae*. 1. Fresh basidiomata (holotype). 2. Fresh basidiomata (CNF 1/5774). 3. Dried basidiomata (holotype). Bars = 5 mm.

of cylindrical or rarely subinflated, mostly thick-walled [walls up to 1.0(–2.0) µm], smooth or diverticulate, up to 6.0(–15) µm wide hyphae; terminal cells cylindrical to clavate, branched to coralloid, thin- or slightly thick-walled, up to 10 µm wide, some of them with apical, ± clavate or subcapitate outgrowth. Clamp connections present and abundant in all tissues. Chemical reactions: all parts of basidioma non-amyloid and non-dextrinoid.

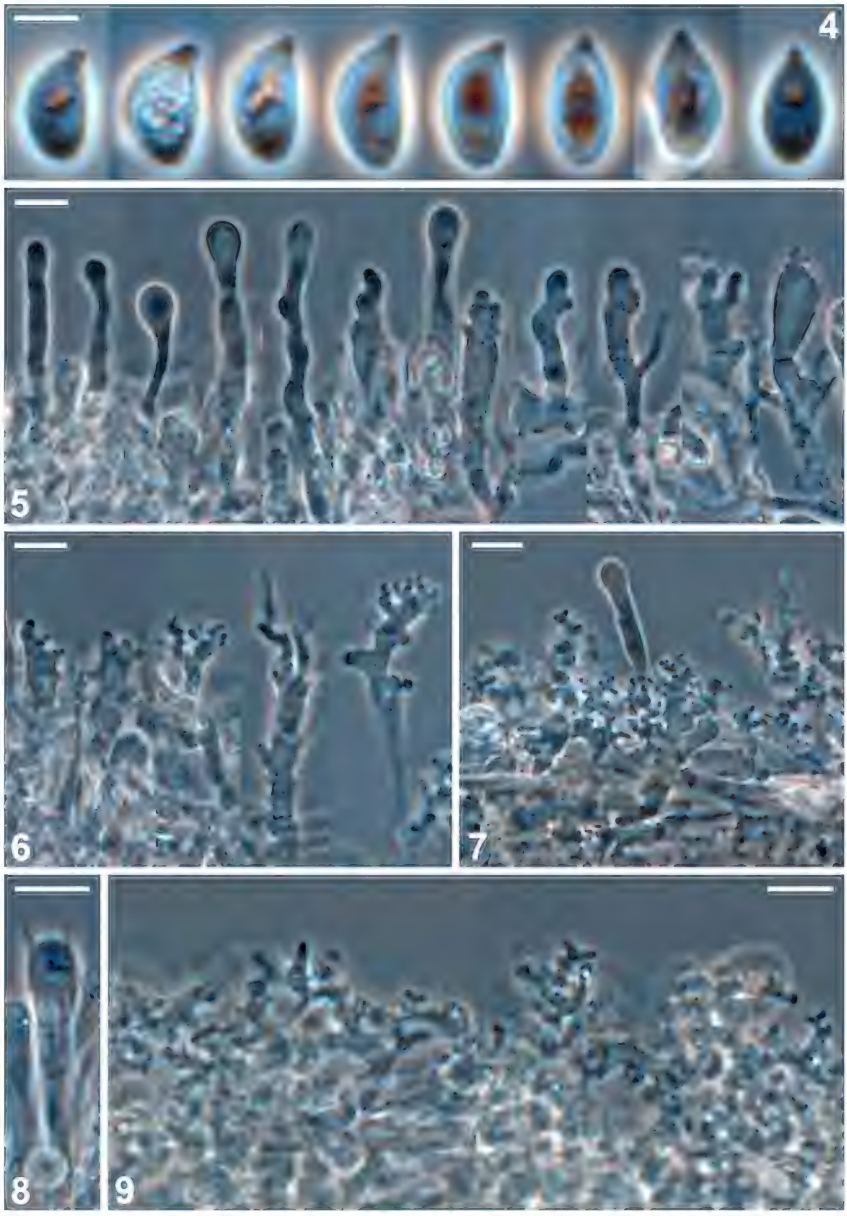
Habitat — On the type locality in the Mediterranean maquis with *Arbutus unedo, Erica arborea, Phillyrea latifolia, Pistacia lentiscus,* and *Myrtus communis;* on hanging, dead stem of *Lonicera implexa.* On additional locality in evergreen park-forest with *Quercus ilex* and *Laurus nobilis;* on dead stem of *Hedera helix.*

Distribution — Known only from two localities in the Mediterranean part of Croatia.

Additional specimen examined: **CROATIA: Dubrovnik–Neretva County,** 10 km NW from Dubrovnik, Trsteno arboretum in Trsteno village, 42°42'43" N 17°58'35" E, alt. 55 m, 13 November 2009; leg. M. Čerkez & A. Mešić (CNF 1/5774, BRNM 736107).

Remarks — *Marasmiellus milicae* is characterized by having a rather small, white pileus, distinctly to strongly eccentric, curved, short, dark stipe, white hymenophore becoming dark brown when dry, (sub)amygdaloid basidiospores, mostly diverticulate cheilocystidia, pileipellis and stipitipellis with strongly developed Rameales-structure, and gelatinized trama. Having those characters, it belongs to sect. *Distantifolii* Singer (Singer 1973). So far, it has been collected only in Croatia.

Several marasmielloid species with similar shape of basidiomata are known in Europe. Among the white colored species, *Gymnopus sphaerosporus* M. Villarreal et al. and *Marasmiellus margaritifer* (Maire) Singer have a white stipe, globose or subglobose basidiospores, and different cheilocystidia (Antonín & Noordeloos 2010). From the white colored species of section *Distantifolii*, *Marasmiellus distantifolius* (Murrill) Singer, known from the USA, Central and South America, especially differs by a pure white stipe, distinctly longer basidiospores ((8.5–)10.2–16 × 3–5.5(–6) µm according to Singer 1973; 11.5–14.5 × 4–5.5 µm according to Pegler 1977, 1997), and smaller cheilocystidia (20–25 × 4.5 µm); *M. corticum* Singer, described from the USA, differs by a shortly radially ferrugineous-brown or ochraceous-brown striped pileus on drying, and smaller ((7–)14–21.5 × (5–)7–9.5 µm), differently shaped cheilocystidia (Singer 1973). *Marasmiellus afer* Pegler, collected in tropical Africa, has a lateral, rarely absent stipe and narrower (8.2–10.8 × 3–4 µm), lacrymoid basidiospores (Pegler 1967, 1977). *Marasmiellus tristis* (G. Stev.) E. Horak (= *Campanella tristis* (G. Stev.) Segedin) from New Zealand differs by its pileus turning glaucous or greenish-gray with age, a dirty yellowish hymenophore when dry, spores with lower Q value (1.4), and the absence of



Figs 4–9. *Marasmiellus milicae* (holotype). 4. Spores. 5, 6. Cheilocystidia. 7. Stipitipellis. 8. Basidium. 9. Pileipellis. All under PhC microscope. Bars: 4 = 5 μm; 5–9 = 10 μm.

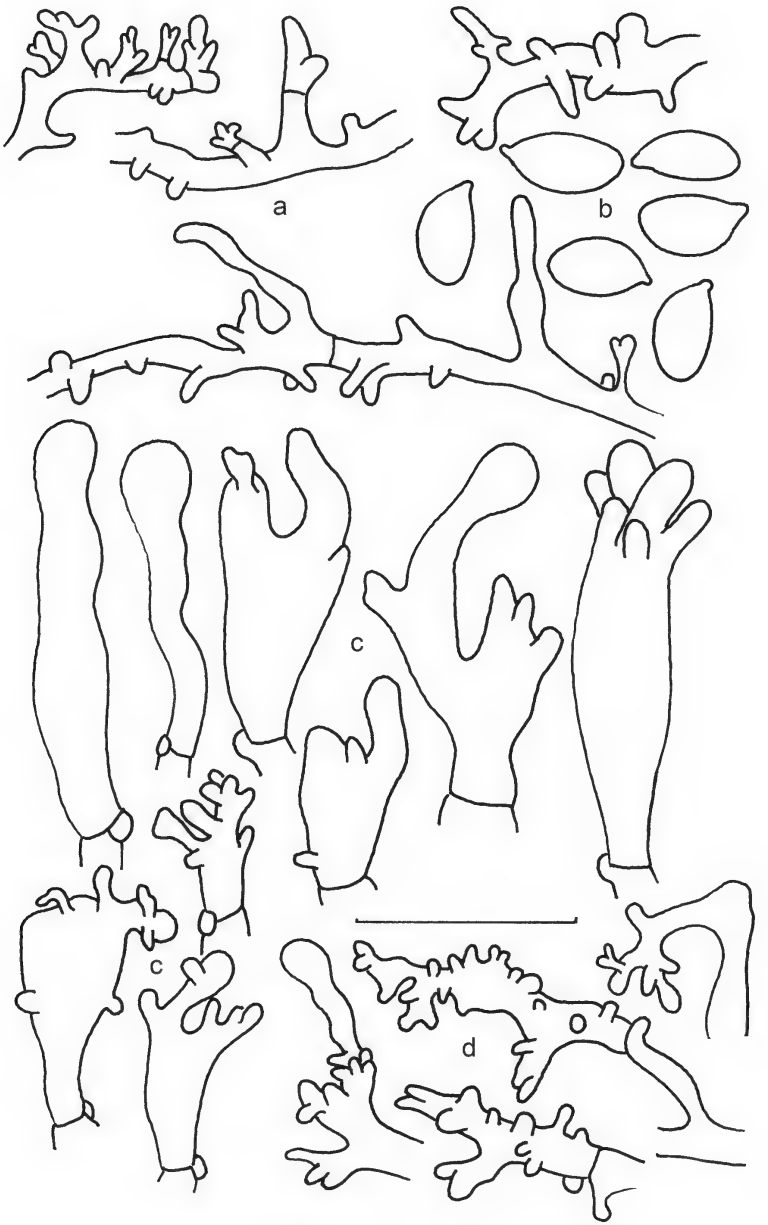


Fig. 10. *Marasmiellus milicae.*
a. Elements of pileipellis. b. Spores. c. Cheilocystidia. d. Elements of stipitipellis. Bar = 20 μm.

branched or coralloid apical projections on cheilocystidia (Segedin 1993). *Marasmius exillimus* Corner, described from Solomon Islands, has a white, pale fuscous striate pileus, a whitish or pallid fuscous stipe, smaller basidiospores (5.5–7.5 × 3.5–4 μm), and a different pileipellis and stipitipellis (Corner 1996).

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Zelodactylaria, an interesting new genus from semi-arid northeast Brazil

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Abstract — During investigation of microfungi on dead plant material in a semiarid region of northeast Brazil, an interesting fungus was collected that is described and illustrated here. *Zelodactylaria verticillata* anam. gen. & sp. nov. is distinguished by macronematous verticillate brown conidiophores with marked sympodially proliferating conidiogenous cells with long cylindrical denticles and solitary obovoid to clavate hyaline 0–1-septate conidia.

Key words — anamorphic fungi, systematic, bark

Introduction

The Brazilian semi-arid zone, called the Caatinga biome, is located almost exclusively in the northeast of the country. This is an expanse of dry land that stretches between 3–17°S and 35–45°W, covers almost 8% of Brazil, and occupies an approximate 900,000 km^2 area. The climate of northeast Brazil is one of the most complex in the world, with an annual rainfall up to 2000 mm along the coast and only 300–500 mm in the semi-arid zone (Giulietti et al. 2006). During a mycological survey of anamorphic fungi from the semi-arid region of Bahia state, a conspicuous fungus was collected that differed morphologically from any previously described anamorphic fungus. It is described here as a new genus.

Materials & methods

Samples of plant material were collected during a mycological survey from the semi-arid region of Bahia, Brazil. Individual collections were placed in paper and plastic bags, taken to the laboratory and treated according to Castañeda (2005). Mounts were prepared in polyvinyl alcohol-glycerol (8 g in 100 ml of water, plus 5 ml of glycerol) and measurements made at a magnification of ×1000. Micrographs were obtained with a Zeiss Axioskop 40.

Taxonomy

Zelodactylaria A.C. Cruz, Gusmão & R.F. Castañeda, **anam. gen. nov.**

MycoBank MB561559

Conidiophora macronematosa, mononematosa, erecta, ramosa, septata, brunnea Cellulae conidiogenae polyblasticae, discretae, sympodialiter prolificantes, denticulatae. Conidia solitaria, clavata, obovoidea usque globosa, septata, hyalina. Teleomorphosis ignota.

Type Species: *Zelodactylaria verticillata* A.C. Cruz et al.

Etymology: Greek, *Zelo-* meaning emulation; *-dactylaria*, referring to a genus of anamorphic fungi.

Colonies on the natural substratum effuse, hairy, brown to olivaceous or black. Mycelium superficial and immersed. Conidiophores macronematous, mononematous, erect, branched, septate, brown or black, smooth or verruculose. Conidiogenous cells polyblastic, discrete, sympodially proliferating, denticulate. Conidial secession schizolytic. Conidia solitary, clavate, obovoid to globose, septate, smooth or verruculose, hyaline. Teleomorph unknown.

Zelodactylaria verticillata A.C. Cruz, Gusmão & R.F. Castañeda, **anam. sp. nov.** Figs. 1 –4

MycoBank MB561560

Conidiophora macronematosa, mononematosa, cum ramis verticillatis (ramis 20–50 × 2.5–4.0 µm), 7- ad 12-septata, brunnea ad basim, 150–220 × 4–10 µm. Cellulae conidiogenae polyblasticae, 10–30 × 2–3 µm denticulatae cum denticulis conspicuis, 1.0–2.5 µm longis . Conidia solitaria, clavata vel obovoidea, 0- ad 1-septata, hyalina, 11–17 × 3–5 µm.

Type: BRAZIL. Bahia: Senhor do Bonfim, "Serra da Maravilha", 12°23′S 40°12′W, on decaying bark of unidentified plant, 22.IX.2006, coll. A.C.R. da Cruz (**Holotype**: HUEFS169132).

Etymology: Latin, *verticillata*, referring to the whorled branches of the conidiophores.

Colonies on the natural substratum, effuse, hairy, brown. Mycelium superficial and immersed. Hyphae septate, branched, smooth, brown. Conidiophores macronematous, mononematous, 7- to 12-septate, erect, straight or flexuous, smooth, 150–220 × 4–10 µm, brown at the base, subhyaline towards the apex; producing verticillate, 20–50 × 2.5–4.0 µm, septate, pale brown to subhyaline branches. Conidiogenous cells polyblastic, discrete, terminal and intercalary, 10–30 × 2–3 µm, subhyaline, with marked sympodial proliferations, with

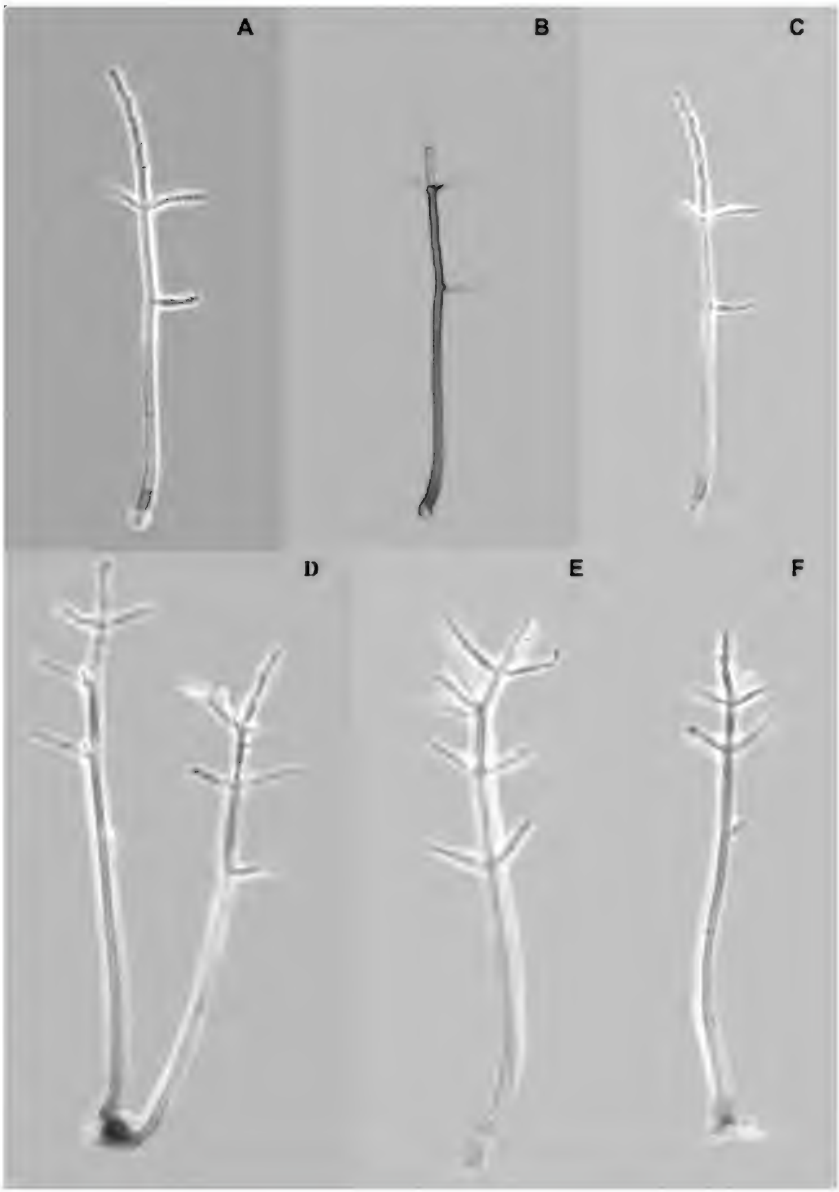

Fig. 1. *Zelodactylaria verticillata*.
A–F. Conidiophores. Scale bar = 50 µm.

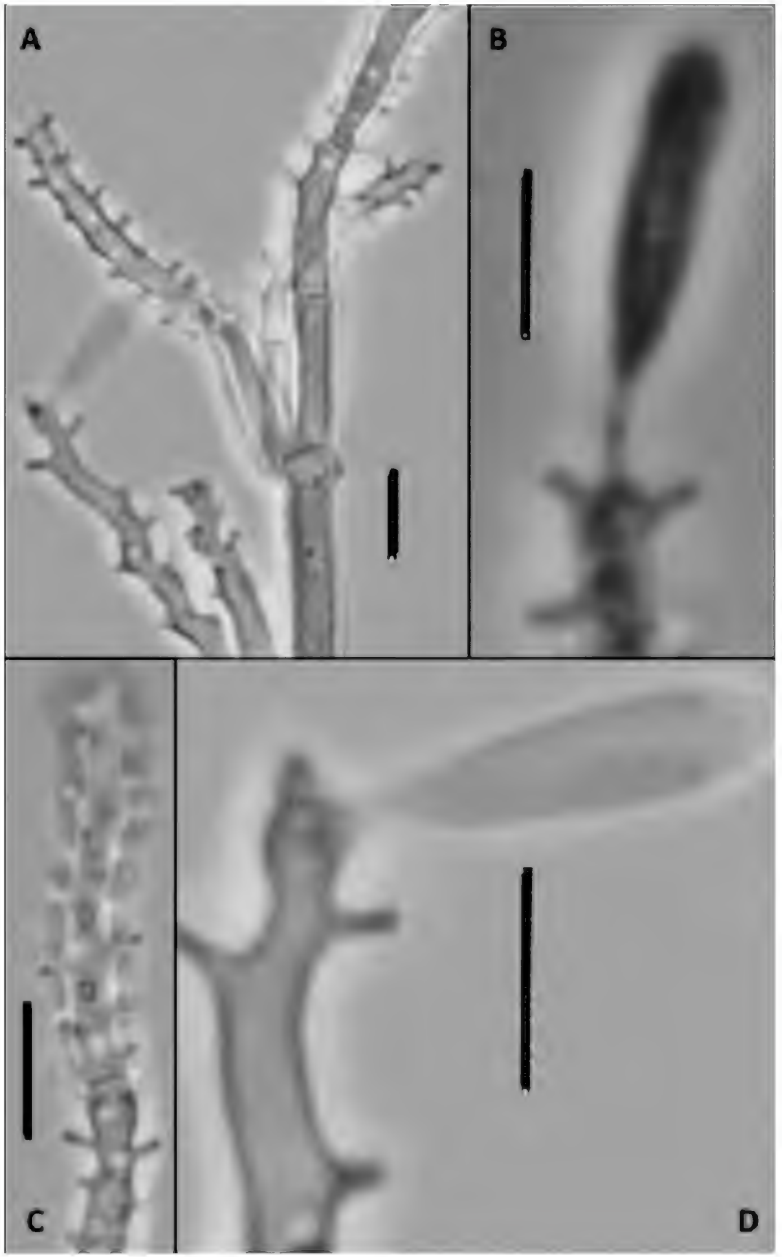

Fig. 2. *Zelodactylaria verticillata*.
A. Conidiophore and conidiogenous cells. B, D. Conidiogenous cells and conidia.
C. Conidiogenous cell. Scale bars = 10 µm.

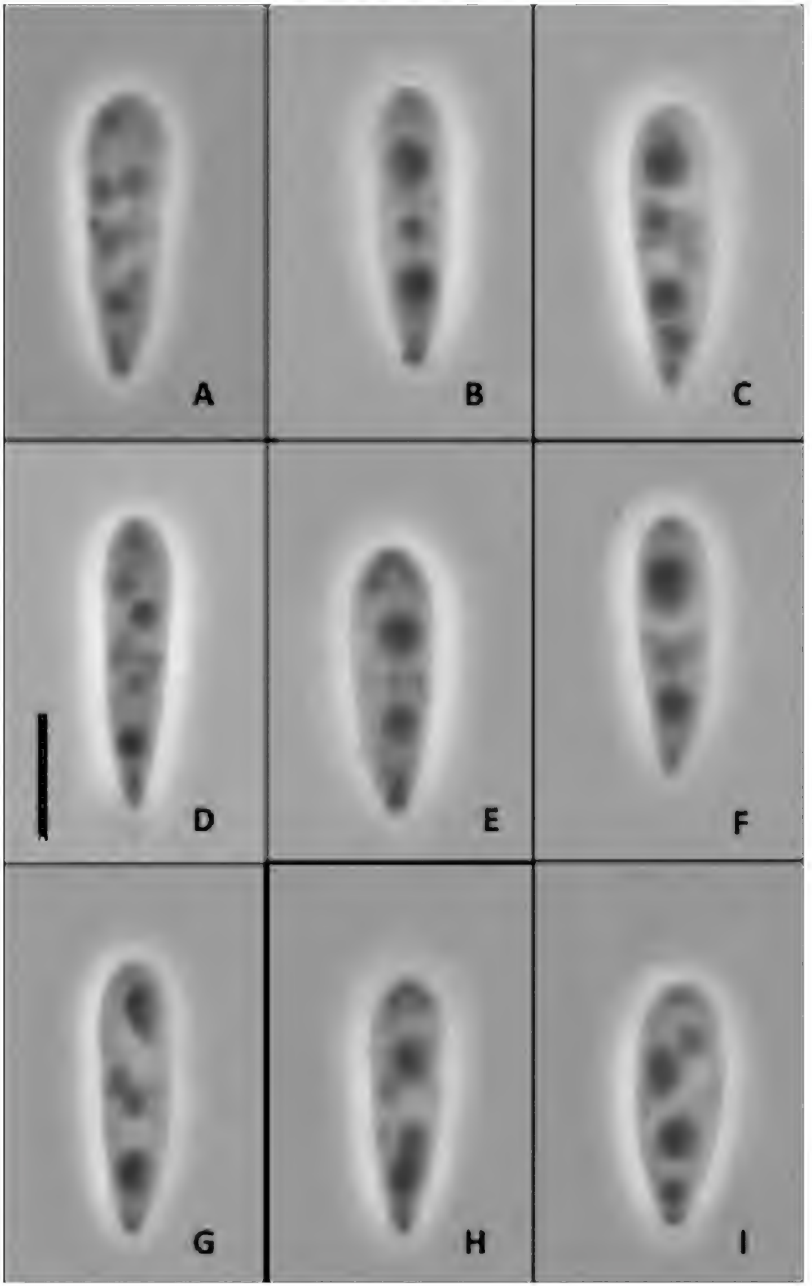

Fig. 3. *Zelodactylaria verticillata*.
A–H. Conidia. Scale bar = 10 µm.

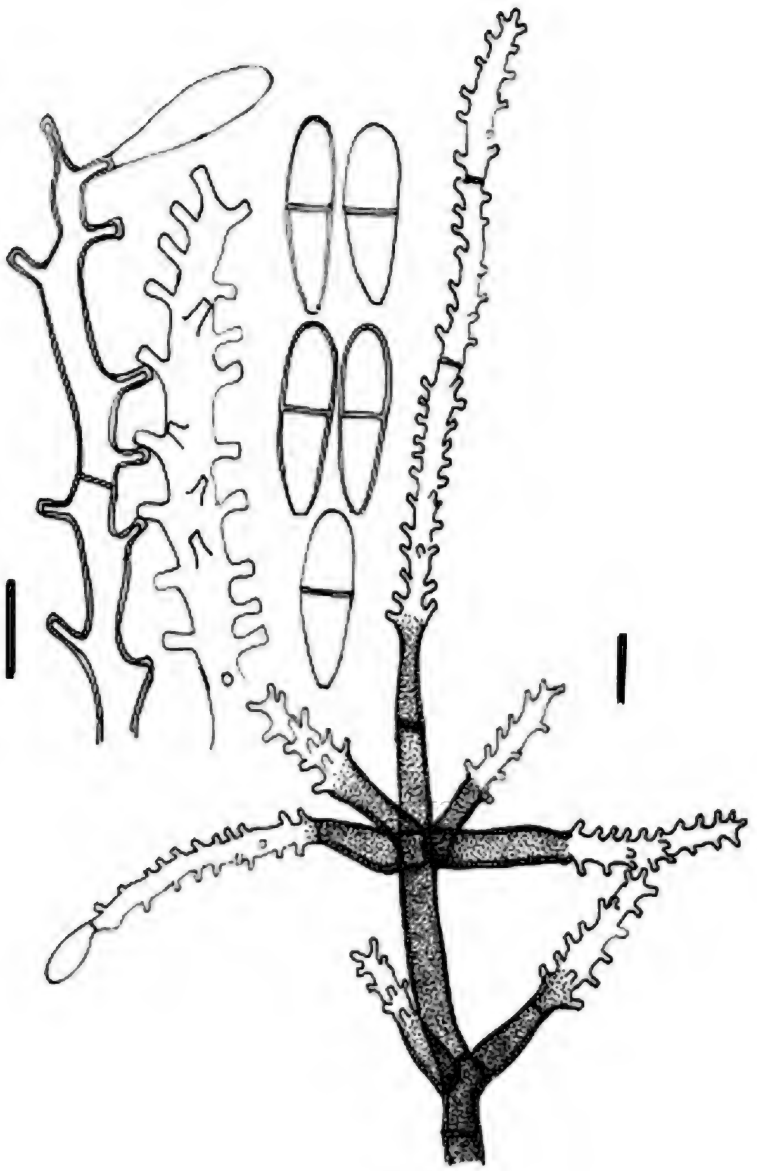

FIG. 4. *Zelodactylaria verticillata.*
Conidiophores, conidiogenous cells and conidia. Scale bars = 10 µm.

conspicuous, shortly cylindrical, 1.0–2.5 µm long, flat-topped denticles. Conidia solitary, clavate to obovoid, truncate at the base, (0–)1-septate, hyaline, guttulate, smooth, dry, 11–17 × 3–5 µm. Teleomorph unknown.

Note: Conidial ontogeny in *Dactylaria* Sacc. (De Hoog 1985, Goh & Hyde 1997, Paulus et al. 2003), *Calcarisporium* Preuss (De Hoog 1974) *Pleurophragmium* Costantin (Matsushima 1985, 1987, 1993, 1995, 1996; Heredia et al. 2007), *Selenosporella* G. Arnaud ex MacGarvie (Castañeda et al. 2009), *Selenosporopsis* R.F. Castañeda & W.B. Kendr. (Castañeda & Kendrick 1991), *Tritirachium* Limber (De Hoog 1972), and *Umbellidion* B. Sutton & Hodges (Sutton & Hodges 1975) can be compared with *Zelodactylaria,* particularly in terms of the sympodial proliferation with denticulate conidiogenous cells. There are, however, clear differences from those genera in respect of the verticillate ramification present in *Zelodactylaria*. The conidiophores in some *Selenosporella* species and the monotypic *Selenosporopsis* are verticillate, but conidia are unicellular, filiform to falcate, produced in short and long denticulate conidiogenous loci respectively. In *Calcarisporium* and *Tritirachium* conidia form on sympodially elongating conidiogenous cells arranged in verticils, but the conidiogenous loci in *Calcarisporium* develop at the top of each branch and in *Tritirachium* are inconspicuous scars in a long geniculate rachis (Kendrick 2003), which also differentiate both genera from *Zelodactylaria*. In *Umbellidion* the conidiogenous cells are arranged in umbels, with several short unthickened, inconspicuous denticles restricted to the apices and the conidia are aseptate.

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A new species of *Ijuhya*, *I. oenanthicola*

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Abstract — *Ijuhya oenanthicola* sp. nov. (*Bionectriaceae*) is described and illustrated based on a collection from *Oenanthe crocata* (*Apiaceae*) in France. The *Acremonium*-like asexual state was obtained in culture.

Key words — *Ascomycota*, *Hypocreales*

Introduction

The situation of Penvenan on the North Coast of Brittany is a privileged one for both its marvellous landscapes and temperate climate. Frost is very rare in winter and summer heat is never overwhelming. Wet zones and marshes have been preserved around the mouths of rivulets, surrounded by rows of old *Salix* trees. Located between Queffioec and the "Anse de Pellinec," such a marsh has proved to be a paradise for fungal diversity. Looking for *Helotiales* on previous year plants or herbaceous stems, our attention was drawn to a tiny, hairy hypocrealean fungus on dead *Oenanthe* remnants. This collection was determined to represent a previously undescribed species. Our specimen was cultured from a single ascospore that produced an *Acremonium*-like asexual state.

Materials & methods

Specimens were examined using the methods described by Rossman et al. (1999). Microscopic observations and measurements were made in water, and the ascospore ornamentation was observed in cotton blue in lactic acid.

Taxonomy

Ijuhya oenanthicola Lechat & Hairaud, **sp. nov.** Plate 1

Mycobank MB 561714

Perithecia subglobosa, apice applanata, ca. 160–235 µm diam, subhyalina vel aurantia, corona subapicalis pilis agglutinatis aurantia, crasse-tunicatis, flexuosis composita, colore in KOH non mutanda. Asci ca. 51 × 9.5 µm, octospori, unitunicati. Ascosporae fusiformes, ca. 15.4 × 3.2 µm uniseptatae, striatae.

Type: France. Brittany: Côtes-d'Armor, Penvenan, Marais de Pellinec, 48°49′53.36″N 3°17′27.23″W, on dead stem of *Oenanthe crocata* L. (*Apiaceae*), 12 Mar 2011, leg. Michel Hairaud, CLL11046 (**Holotype**, LIP; ex-type culture CBS 129747).

Etymology: The epithet refers to the substratum on which this species was collected.

Perithecia scattered on stem of *Oenanthe*, superficial, subglobose, (160–) 200–220(–235) µm diam, at first white, becoming pale orange to dark orange, collapsing laterally, not changing color in 3% KOH or lactic acid, with orange, fasciculate, thick-walled hairs, agglutinated to form teeth arranged in a stellate fringe around upper margin of perithecia, teeth up to 60 µm wide at base. Hairs flexuous, thick-walled, 20–150(–165) µm long, 2–3 µm wide, rounded at tip, hyaline, smooth, cylindrical with wall 1–1.7 µm thick, aseptate. Perithecial wall 18–23 µm thick, composed of two regions: outer region 10–14 µm wide, of globose to ellipsoidal, 3.5–6 × 2–3.5 µm cells, with yellow to orange wall 1.5–2.2 µm thick; inner region 5–8 µm wide, of elongate, flattened cells 5–11 × 1.5–2 µm, with hyaline wall 0.5–1 µm thick. Asci (40–)45–55(–60) × (6–) 8–10(–12) µm (^{m} = 51 × 9.5 µm, n = 20), clavate, apices flat to rounded, without ring, containing irregularly biseriate ascospores, completely filling each ascus when 8-spored, numerous asci in which 1–3 or even 4 of 8 ascospores aborted. Ascospores (12–)14–16.5(–18) × (2.5–)3–3.5(3.8) µm (^{m} = 15.4 × 3.2 µm, n = 30), fusiform, rounded at ends, 1-septate, hyaline, striate, constricted at septum.

Anamorph: *Acremonium*-like

In Culture: After two weeks at 25°C on Difco PDA containing 5mg/L streptomycin, colony 3–3.5 cm diam, spreading a reddish brown coloration in medium, mycelium white to pale yellow, producing an Acremonium-like culture at margin of colony, composed of monophialidic conidiophores, 42–55 µm long, 3–4 µm diam with 1-septum at base, arising from smooth hyphae 2.5–3.8 µm diam, producing cylindrical to widely ellipsoidal conidia (3.5–) 4–6.5(–7) × 2–4 µm (^{m} = 6.1 × 3.2 µm, n = 30), hyaline, smooth, non-septate, with a basal abscission scar.

Key to *Ijuhya* species with fasciculate hairs

(modified from Lechat & Courtecuisse 2010)

1. Hairs 200–300 µm long; ascospores (24–)30–60(–110) × 4–7(–8) µm, striate; ascomata pale yellow ... ***I. peristomialis*** (Berk. & Broome) Rossman & Samuels

1. Hairs averaging <200 µm long **2**

2. Ascospores averaging <12 µm long **3**

2. Ascospores averaging >12 µm long **4**

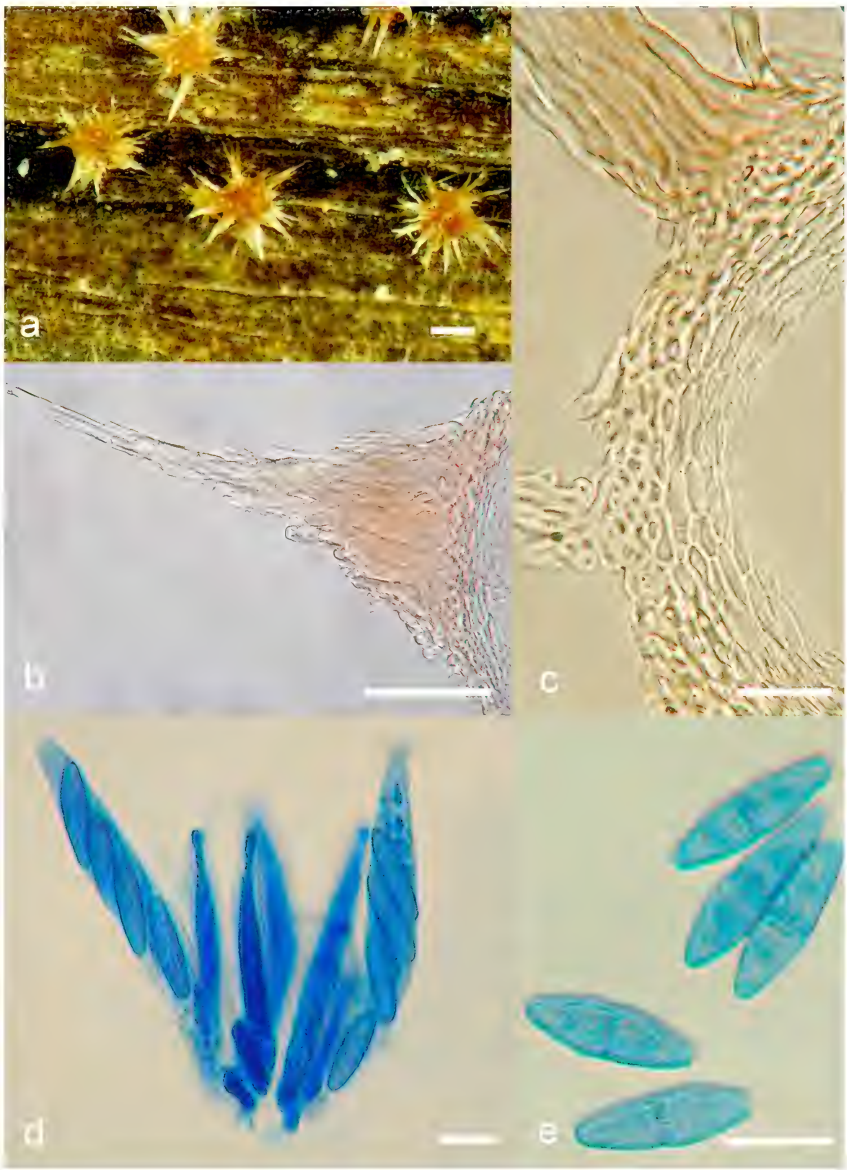

Plate 1. *Ijuhya oenanthicola* (based on holotype material). **a.** Perithecia. **b.** Fasciculate hairs. **c.** Median section of perithecial wall. **d.** Asci. **e.** Ascospores. Scale bars: a = 200 µm; b = 50 µm; c = 15 µm; d–e = 10 µm. Additional photos at http://www.ascofrance.fr.

3. Ascospores (8.5–)9.5–11.5(–12.5) × 2.8–3.2(–3.5) µm, striate;
ascomata pale yellow to brownish-orange,
hairs 28–80 × 2–2.5(–3) µm ..... ***I. equiseti-hyemalis*** Lechat & Baral

3. Ascospores 6–8(–9) × 3–4 µm, spinulose; ascomata orange-yellow,
hairs 150–200 × 3–4 µm ..... ***I. dentifera*** (Samuels) Rossman & Samuels

4. Ascospores striate,..... .5

4. Ascospores spinulose 14.5–20 × (2.5–)3–5(–5.4) µm, ascomata brownish-orange,
hairs 30–50 µm long..... ***I. parilis*** (Syd.) Rossman & Samuels

5. Asci (4–6–)8-spored, ascospores (12–)14–16.5(–18) × (2.5–)3–3.5(3.8) µm;
hairs 20–140 µm × 2.5–3 µm, non-septate, ascomata dark orange ***I. oenanthicola***

5. Asci constantly 8-spored 6

6. Ascospores (10.5–)11–13(–14) × 2.5–3.5 µm; ascomata dark orange,
hairs up to 160 µm long..... ***I. antillana*** Lechat & Courtec.

6. Ascospores (19–)21–28 × 3.5–4.5µm; ascomata dull orange,
hairs up to 100 µm long..... ***I. chilensis*** (Speg.) Rossman & Samuels

Discussion

In the genus *Ijuhya* Starbäck (*Bionectriaceae*) based on the type *I. vitrea* Starbäck, ten species were recognized by Rossman & Samuels (1999). Since then, *Ijuhya hongkongensis* J. Fröhl. & K.D. Hyde (Fröhlich & Hyde 2000), *I. hubeiensis* Y. Nong & W.Y. Zhuang (Zhuang et al. 2007), *I. equiseti-hyemalis* (Lechat & Baral 2008), and *I. antillana* (Lechat & Courtecuisse 2010) were added.

Ijuhya oenanthicola differs from other *Ijuhya* species bearing fasciculate hairs in having < 200 µm long non-septate hairs, > 14 µm long striate ascospores, numerous asci with 1–3 (or even 4) out of 8 ascospores aborted, and its occurrence on *Oenanthe crocata*.

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Two new species of *Hyphoderma* (*Agaricomycetes*) from India

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Abstract – Two new corticioid species, *Hyphoderma bicystidiatum* and *H. subglobosum,* are described from Kangra Hills in Himachal Pradesh.

Key words – northwestern Himalaya, angiosperm host

While conducting fungal forays in district Kangra of Himachal Pradesh, India, Priyanka collected two interesting corticioid fungi that were referred to *Hyphoderma* based on detailed macroscopic and microscopic comparisons (Eriksson & Ryvarden 1975, 1976; Martin & Gilbertson 1977; Rattan 1977; Burdsall & Nakasone 1983; Dhingra 1989, Dhingra & Singla 1993, Natarajan & Kolandavelu 1998, Dhingra & Singh 2009, Dhingra et al. 2009, Singh et al. 2010). The specimens could not be assigned to any known taxa and are here described as two new species. Samples of both collections were sent to Prof. Nils Hallenberg, who also supported the concept of referring the new species to *Hyphoderma*.

Hyphoderma bicystidiatum Priyanka & Dhingra, **sp. nov.** Figs 1–7

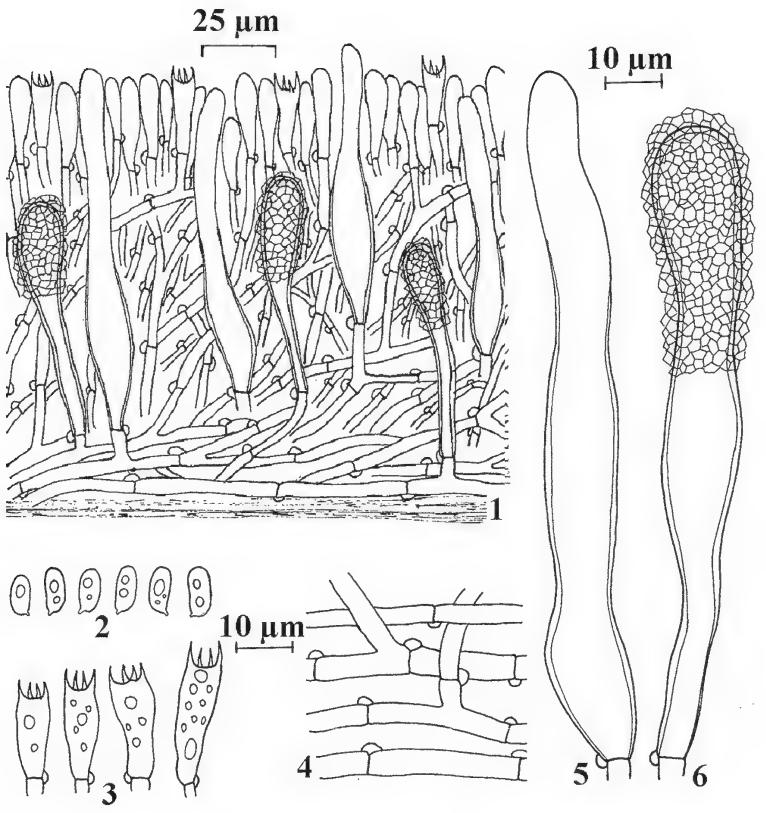
MycoBank MB 560555

Differs from *Hyphoderma pallidum* in the presence of thick-walled clavate encrusted cystidia.

Type: India, Himachal Pradesh: Kangra, Shiv Nagar, on decaying angiospermous branches, 25 August 2009, Priyanka 4298 (PUN, **holotype**).

Etymology: The epithet refers to the presence of two types of cystidia.

Basidiocarp resupinate, effused, adnate, up to 250 µm thick in section; hymenial surface smooth to somewhat tuberculate under the lens, yellowish white to pale yellow; margins thinning out, concolorous but paler. Hyphal system monomitic; generative hyphae up to 5.5 µm wide, ordinarily branched, with clamps at all



Figs 1–6. *Hyphoderma bicystidiatum*: microscopic structures.
1. vertical section through basidiocarp; 2. basidiospores; 3. basidia; 4. generative hyphae; 5. smooth cystidium; 6. thick-walled encrusted cystidium.

septa, thin-walled; hyphae loosely arranged and parallel to the substrate in the basal region and gradually becoming vertically oriented towards the hymenium. Cystidia of two kinds: (i) 82.0–122.0 × 12.3–13.8 µm, tubular, basally widened and somewhat thick-walled, apically thin-walled, smooth; (ii) 69.0–110.0 × 11.0–13.8 µm, clavate to subclavate, thick-walled, encrusted in the apical third part. Basidia 13.6–22.3 × 6.2–7.5 µm, clavate to subclavate, 4-sterigmate, with oily contents and basal clamp; sterigmata up to 4.8 µm long. Basidiospores 6.2–8.8 × 3.0–4.1 µm, subellipsoid to suballantoid, thin-walled, smooth, inamyloid, acyanophilous, with oily contents.

Remarks—*Hyphoderma bicystidiatum* resembles *H. argillaceum* and *H. pallidum* in having basally widened cystidial elements and resinous matter in the

Fig. 7. *Hyphoderma bicystidiatum*: basidiocarp showing hymenial surface.

context. The new species is also similar to *H. pallidum* in the shape of basidiospores. It differs from both, however, in having clavate, thick-walled, encrusted, cystidia. The new combination of features suggests a species of its own.

Hyphoderma subglobosum Priyanka & Dhingra, **sp. nov.** Figs 8–13

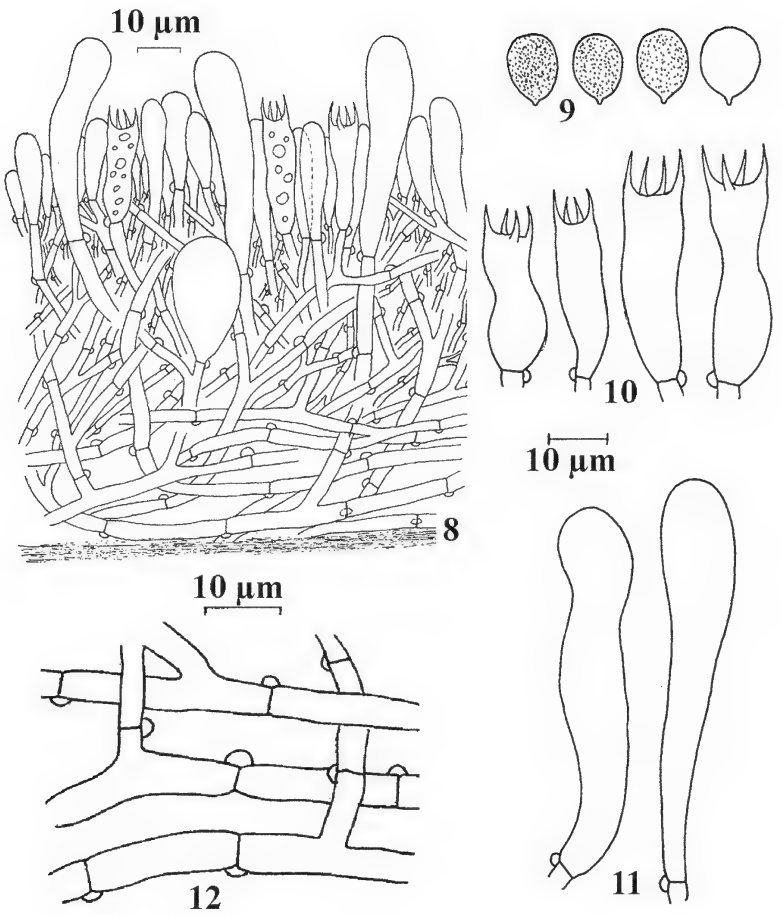
MycoBank MB 560581

Differs from *Hyphoderma clavigerum* in subglobose basidiospsore shape.

Type: India, Himachal Pradesh: Kangra, about 3 km from Nurpur towards Sulyali, on decaying angiospermous branches, 26 August 2009, Priyanka 4299 (PUN, **holotype**).

Etymology: The epithet refers to the shape of basidiospores.

Basidiocarp resupinate, effused, adnate, up to 170 µm thick in section; hymenial surface smooth to somewhat tuberculate under the lens, grayish yellow to grayish orange; margins thinning out, indeterminate, concolorous but paler. Hyphal system monomitic; generative hyphae up to 5.6 µm wide, ordinarily branched, with clamps at all septa, thin-walled; hyphae almost parallel to the substrate in the basal zone, gradually becoming vertical and densely intertwined in the subhymenial and hymenial zones. Cystidia 50.0–69.0 × 8.0–11.8 µm, clavate, smooth, thin-walled. Basidia 22.0–34.0 × 6.8–10.0 µm, clavate to subclavate, mostly constricted at the middle, with oily contents, 4-sterigmate,



Figs 8–12. *Hyphoderma subglobosum*: microscopic structures.
8. vertical section through basidiocarp; 9. basidiospores; 10. basidia;
11. cystidia; 12. generative hyphae.

with basal clamp; sterigmata up to 6.8 µm long. Basidiospores 11.8–13.1 × 8.0–10.0 µm, subglobose to broadly ellipsoid, thin-walled, smooth, inamyloid, acyanophilous, with grainy contents.

Remarks—The subglobose spores and the clavate cystidia with light refracting content are diagnostic characters. *Hyphoderma subglobosum* is close to *H. clavigerum* in having similarly shaped cystidial elements but differs considerably in the shape of basidiospores i.e. subglobose in comparison to

Fig. 13. *Hyphoderma subglobosum*: basidiocarp showing hymenial surface.

suballantoid. The combination of subglobose spores and clavate cystidia with light refracting content suggests that the collection represents a new species.

Acknowledgements

The authors thank Prof. Nils Hallenberg (Gothenburg, Sweden) for valuable suggestions and peer review; Prof. B.M. Sharma, Department of Plant Pathology, COA, CSKHPAU, Palampur, H.P., India for peer review; Head, Department of Botany, Punjabi University, Patiala, is thanked for providing research facilities.

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New records of noteworthy gasteroid fungi from Pakistan

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Abstract — Gasteroid fungi were collected during the course of fieldwork in Pakistan. Here, we report on two species, *Bovistella japonica* and *Lycoperdon excipuliforme*, from the Himalayan moist temperate forest of the Khyber Pakhtunkhwa Province, each a new record for the country. *Phellorinia herculeana* represents a new record from Cholistan Desert of the Punjab Province. For each taxon reported, detailed macro- and microscopic descriptions are presented.

Key words — Biomes, Nathia gali, taxonomy

Introduction

The wide range of habitats in Pakistan that extends from coastal regions along the Arabian Sea to arid scrubland in the Punjab region and to the high altitude forests and tundra of the Himalayas supports a rich diversity of fungi. One group, the gasteroid fungi, has been documented from the nation in several publications (Long & Ahmad 1947, Ahmad 1952, Yoshimi & Hagiwara 1992, Khalid & Iqbal 1996, Ahmad et al. 1997, Sultan et al. 2001, Razzaq & Shahzad 2004, 2007, Iqbal et al. 2006, Sultana et al. 2007, Moreno et al. 2009). In total, 112 species representing approximately 30 genera have been reported from Pakistan. Although a considerable number of taxa are known, it is likely that the present accounting of Pakistani gasteroid fungi is far from complete, as a large portion of the country has been sampled only in a cursory manner, if at all.

In this study, we collected gasteroid fungi in two different biomes of Pakistan — the moist temperate forest of the Himalayas and scrubland of the Cholistan Desert. The Himalayan moist temperate forest extends from the Murree Hills of the Ayubia National Park to the Swat Valley (Khyber Pakhtunkhwa province), along the outer range of the Himalayas at an altitude of around 1850–2500 m (Ahmad et al. 2006). The Cholistan Desert, which covers a 26,000 km^2 area

in southwest Punjab province, is among the hottest and driest regions in the country (Akbar et al. 1996).

Here we report on three gasteroid species from Pakistan: *Bovistella japonica* and *Lycoperdon excipuliforme* were collected in the Himalayan moist temperate forest and represent new records for the country, while *Phellorinia herculeana* (previously reported from Pakistan as *P. inquinans*) represents a new record for the Cholistan Desert.

Materials & methods

Observations of microscopic characters (e.g., spore dimensions, eucapillitium, peridial hyphae) were made under a light microscope (at 1000× magnification) on material mounted in lactophenol and 5% KOH medium. Glebal color and macroscopic peridial features were observed using Meiji stereomicroscope, and illustrations were made with the help of a camera lucida.

The specimens have been deposited in gasteroid fungal collection of LAH Herbarium, Department of Botany, University of the Punjab, Lahore, Pakistan.

Taxonomy

Bovistella japonica Lloyd, Mycol. Writ. 2: 281 (1906) FIG. 1

GASTEROCARP 55–85 mm diam. × 85 mm high, globose to subglobose, white when young, turning reddish brown with age. STERILE BASE well developed, up to 30 mm high, spongy, chambered, basally attached to the substratum by well developed, thick mycelial strand, having white, branched rhizomorphs heavily encrusted with soil particles; ostiole lacking; dehiscence irregular, fruiting body breaking apart apically. PERIDIUM < 1mm thick. EXOPERIDIUM white when young, becoming dull brown with age, thin, formed of granular matter which covers the endoperidium, persistent. ENDOPERIDIUM entirely covered by exoperidium even in older specimen, light brown, thin, papery. GLEBA brown, cottony, compact.

BASIDIOSPORES subglobose to ovoid, 4.0–5.5 × 3.8–4.7 µm, hyaline to greenish in KOH, pedicellate, pedicels short to very long, up to 19 µm long. CAPILLITIUM *Bovista* type, well developed, separate units having a thick main axis (≤ 20 µm diam.), thick walled (walls ≤ 2.85 µm), branches originating from the main axis, 1.2–6.0 µm diam. (walls ≤ 1.0 µm), with attenuate tips. EXOPERIDIUM composed of subglobose to irregular sphaerocysts. ENDOPERIDIUM composed of hyaline, unbranched, and aseptate hyphae. STERILE BASE composed of pigmented, thick walled, branched, and aseptate hyphae.

MATERIAL EXAMINED: **PAKISTAN: KHYBER PAKHTUN KHWA**, Nathia Gali, ca. 2500m (8205 ft) a.s.l., solitary, on ground, under *Pinus wallichiana* A.B. Jacks., in Himalayan moist temperate forest, 13 Sep. 2006, N. Yousaf NYG208 (LAH 130906).

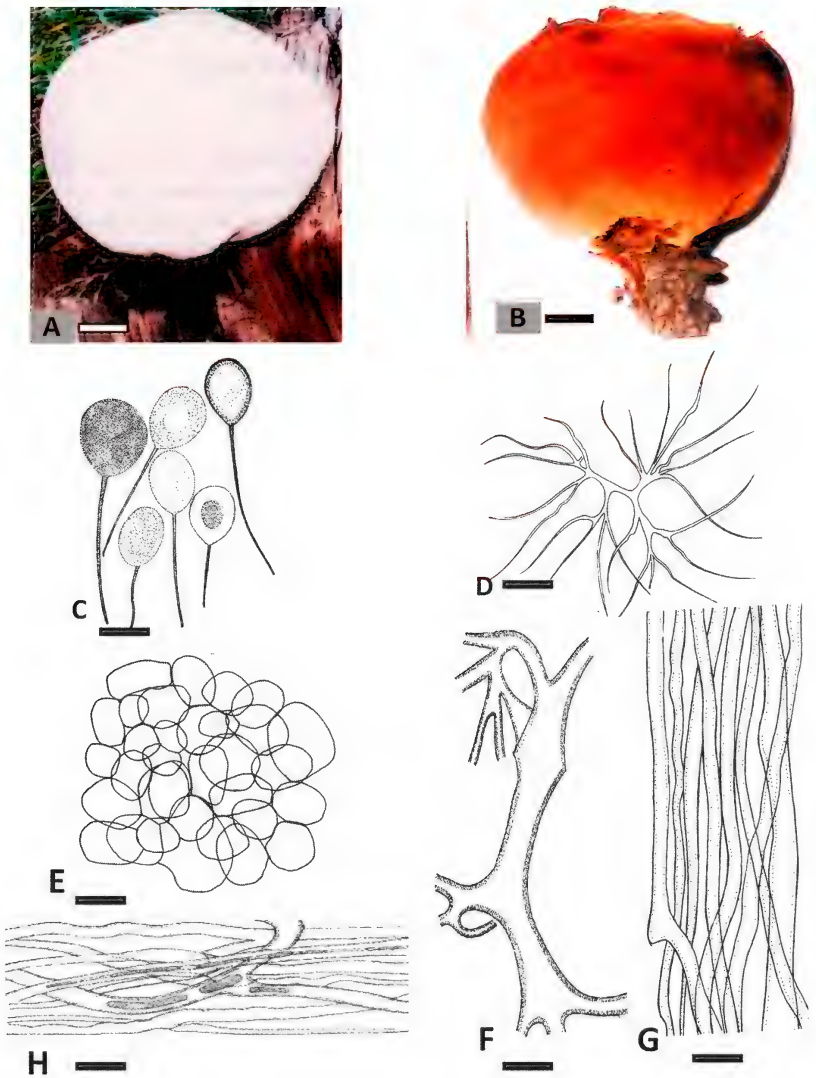

Fig. 1: *Bovistella japonica*. A. Young gasterocarp. B. Mature gasterocarp. C. Pedicellate basidiospores. D. Typical *Bovista* type capillitial units. E. Exoperidial elements (sphaerocysts). F. Thick, main axis of capillitial threads. G. Aseptate endoperidial hyphae. H. Hyphae of sterile base. Scale bars: A, B = 1.5 cm; C = 2.5 µm; D = 60 µm; E = 3 µm; F = 20 µm; G, H = 10 µm.

Comments: *Bovistella* was first proposed by Morgan in 1892, and the genus is distributed worldwide. *Bovistella japonica* is characterized by the presence

of short, separate capillitial units, pedicellate spores, and sterile base. The combination of *Bovista*-like capillitial units and *Lycoperdon*-like sterile base places the species in the intermediate genus, *Bovistella,* along with *B. ohiensis* (Ellis & Morgan) Morgan, *B. radicata* (Durieu & Mont.) Pat., *B. lycoperdoides* (Schwein.) Lloyd, *B. paludosa* (Lév.) Pat., and *B. ammophila* (Lév.) Lloyd. Among these taxa, *B. japonica* is more closely allied with *B. ohiensis* and *B. paludosa*, which also share the same general spore dimensions (~4–5 µm). *Bovistella japonica* can be distinguished by its more compact sterile base. This species is a new addition to the mycoflora of Pakistan.

Lycoperdon excipuliforme (Scop.) Pers., Syn. meth. fung.: 143 (1801) FIG. 2

GASTEROCARP elliptical to turbinate, 30–50 mm diam. × 50 mm high; spore case broad, globose to subglobose; ostiole present, irregular. STERILE BASE present, and forming a well-developed pseudostipe, attached to the substrata by well developed rhizomorphs, these white, branched, thick, and heavily encrusted with soil particles, and woody debris. PERIDIUM layered, thickness ≤ 1 mm. EXOPERIDIUM dull brown, fragile, in the form of granules which later slough off. ENDOPERIDIUM dull, golden brown, papery. GLEBA dark brown, pulverulent.

BASIDIOSPORES globose to subglobose, dark brown, 5.3–6.7 µm diam. (4.4–5.5 µm excluding ornamentation), verrucose (verrucae ≤ 1 µm), numerous sterigmal remnants present in mounts, these up to 35.6 µm long. CAPILLITIUM *Lycoperdon*-type, capillitial threads 1.14–6.8 µm diam., branched, (dichotomous when occurring), thick-walled (walls ≤ 2 µm), sub undulated to undulate, aseptate, having slit-like pores, with attenuate tips. PARACAPILLITIUM absent. EXOPERIDIUM composed of subglobose sphaerocysts. ENDOPERIDIUM composed of unbranched, septate hyphae.

MATERIAL EXAMINED: **PAKISTAN**: **KHYBER PAKHTUN KHWA**, Nathia Gali, ca. 2500m (8205 ft) a.s.l., solitary, on ground, under bamboo tree, in Himalayan moist temperate forests, 26 Aug. 2006, N. Yousaf NYG209 (LAH 268006).

COMMENTS: Commonly called the "pestle puffball," *L. excipuliforme* usually fruits in groups from summer to autumn in coniferous and deciduous woodlands (Jordan 2006). It is characterized by large basidiomes consisting of an upper spore case and long pseudostipe and by the presence of slit-like pores in its capillitial threads. *Lycoperdon perlatum* Pers., which also develops the same reticulate pattern over the endoperidial surface as the larger verrucae slough off, differs in its smaller stature and the absence of a well-developed elongated pseudostipe. *Lycoperdon excipuliforme* is new to the mycoflora of Pakistan.

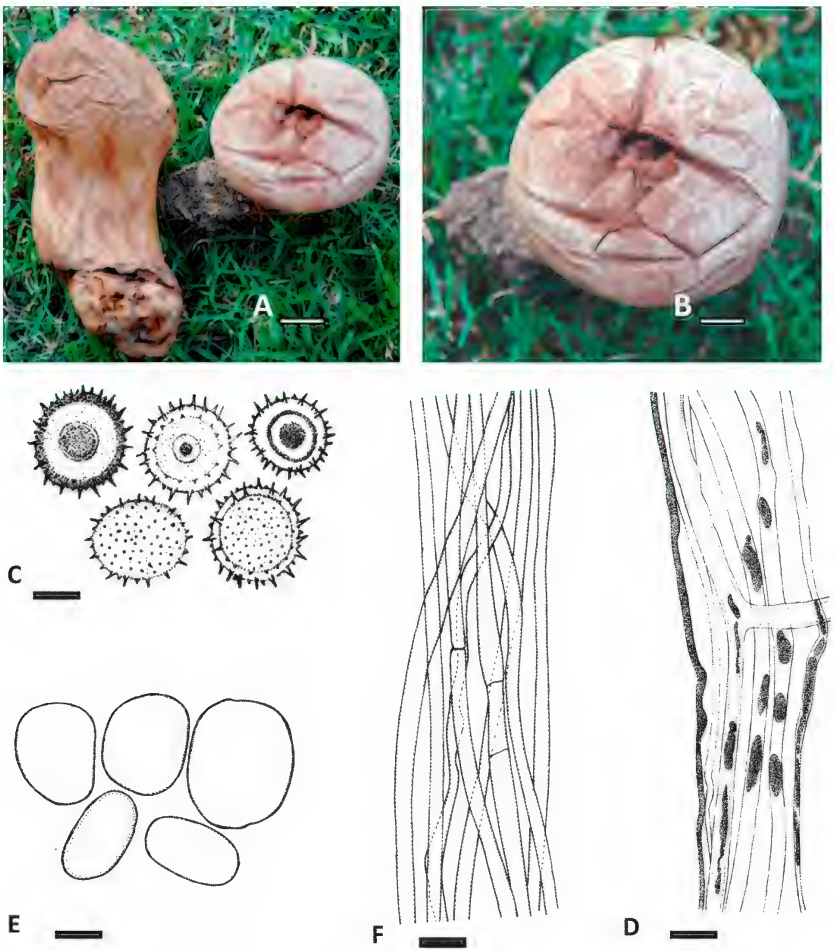

Fig. 2: *Lycoperdon excipuliforme.* A. Gasterocarps. B. Ostiole (arrow). C. Verrucose basidiospores. D. Capillitial threads. E. Exoperidial elements (sphaerocysts). F. Endoperidial hyphae. Scale Bars: A, B = 0.8 cm; C = 2.5 µm; D, F = 10 µm; E = 3 µm.

Phellorinia herculeana (Pers.) Kreisel, Česká Mykol. 15: 196 (1961) Fig. 3

= *Phellorinia inquinans* Berk., London J. Bot. 2: 521 (1843)

Gasterocarp consisting of spore case, stipe, and bulbous base, ≤ 85 mm tall; spore case ≤ 50 mm diam. × 35 mm high, basal portion encrusted with soil particles; dehiscing as apical portions wear away to expose the powdery spore mass. Stipe woody, continuous with spore case, not uniform, having a wide,

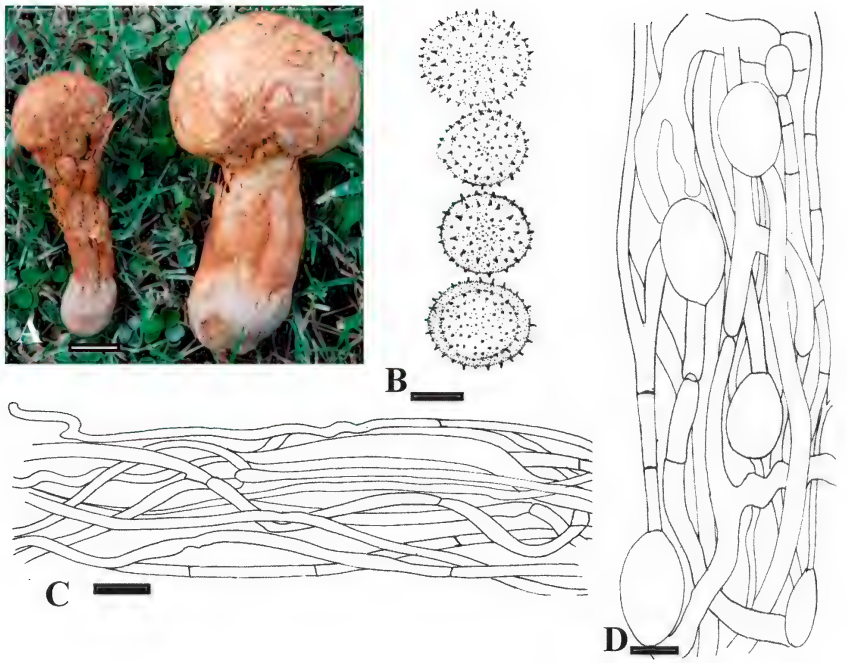

Fig. 3: *Phellorinia herculeana*. A. Gasterocarps. B. Verrucose basidiospores. C. Endoperdial hyphae. D. Exoperidial hyphae with globular elements. Scale Bars: A = 1.2 cm; B = 3 µm; C, D = 10 µm.

bulbous base, and tapering toward the apex, with parallel, broad ridges and furrows running from apex to base, and permanent scales scatter intermittently; rhizomorphs not observed. Exoperidium off-white when immature, pale yellowish to brown with age, thick, entirely covered with hard scales of variable sizes. Endoperidium continuous with stipe, pale yellow. Gleba pulverulent, brown.

Basidiospores globose to subglobose, apedicellate, hyaline, 4.5–8.0 µm diam., verrucose (verrucae < 1 µm long). Capillitium hyaline, rarely present in mounts. Exoperidium composed of branched, hyaline hyphae, with inflated, globose elements at their tips. Endoperidium composed of thin, hyaline, branching, septate hyphae.

Material examined: **PAKISTAN**: **Punjab**, Bahawalpur, Rahim Yar Khan, ca. 115 m (380 ft) a.s.l., Cholistan Desert, solitary, on ground, 29 Sep. 2006, N. Yousaf NYG210 (LAH 290900).

Comments: *Phellorinia* was erected by Berkeley in 1843 with *P. inquinans* Berk. as the type species. It is a monotypic genus that has been reported from

different regions of Pakistan by Ahmad (1952). *Phellorinia herculeana* is widely distributed across arid to semi-arid regions of all continents, including countries such as Australia (Fuhrer 2005, Williams & Woinarski 1997), Pakistan (Ahmad 1952, Ahmad et al. 1997), Spain, and Yemen (Kreisel & Fatimi 2004).

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Pseudocercosporella stellariicola, a new hyphomycete occurring on *Stellaria aquatica*

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Abstract — A new cercosporoid fungus, *Pseudocercosporella stellariicola*, was collected on leaves of *Stellaria aquatica* (*Caryophyllaceae*). This fungus is described, discussed, and compared with other similar hyphomycetes occurring on *Stellaria* and related genera: *Cercosporella echinulata*, *Phacellium episphaerium*, *Pseudocercosporella cerastii*, and *Ps. woronovii*. The phylogenetic relationship of *Ps. stellariicola* and other *Pseudocercosporella* species within *Mycosphaerellaceae* is also inferred from the ITS rDNA sequence data.

Key words — anamorphic *Mycosphaerella*, imperfect fungi, plant pathogen

Introduction

As a result of extensive studies conducted on the mycobiotas of cercosporoid fungi in Korea from 1990 to 2000, Shin & Kim (2001) published a monograph comprising 127 species in 14 genera on 151 species of host plants. Although *Stellaria aquatica*, one of the most common wild plants in Korea, was frequently found infected with *Phacellium episphaerium*, a previously undescribed leaf-spotting hyphomycete has also been found on this plant since 2004. This fungus differed from other similar hyphomycetes known to occur on *Stellaria* and related genera. It was well characterized by colorless conidiophores in dense fascicles, inconspicuous, unthickened conidial scars and solitary, hyaline conidia. These features agree with the concept of *Pseudocercosporella* proposed by Braun (1990), and this hyphomycete is identified and characterized as a novel species in that genus.

Materials & methods

For microscopy, free-hand sections of fresh materials were mounted in water. Morphological characteristics were measured using a model BX51 microscope (Olympus, Tokyo, Japan), and photographed using an Axio imager microscope (Carl

Zeiss, Göttingen, Germany). The voucher specimens were deposited in the Korea University herbarium (KUS).

Monoconidial isolates were deposited at the Korean Agricultural Culture Collection (KACC) of the National Academy of Agricultural Science in Korea and the Centraalbureau voor Schimmelcultures (CBS) in the Netherlands. Genomic DNA was extracted using mycelia harvested from colonies following a previously described method (Lee & Taylor 1990). The ITS rDNA region was amplified using universal primers ITS1/ITS4 (White et al. 1990). The PCR amplicons were purified using a MultiScreen HTS™ PCR filter plates (Millipore, Carrigtwohill, Cork, Ireland) and sequenced using an ABI Prism™ 377 automatic DNA sequencer (Applied Biosystems, Foster City, CA, USA) with a BigDye™ cycle sequencing kit v. 3.1 (Applied Biosystems). The raw sequences were edited using the DNASTAR computer package v. 5.05 (Lasergene, Madison, WI, USA). The ITS rDNA sequences obtained from three isolates (KACC42363, KACC42395, and CPC11297) have been deposited in GenBank with accession numbers EF600945, EF600956, and GU214693, respectively. For phylogenetic analysis, available ITS rDNA sequences of *Pseudocercosporella* spp. and closely related taxa belonging to *Mycosphaerellaceae* were retrieved from GenBank. The sequences were aligned with ClustalX v. 2.1 (Larkin et al. 2007). The Bayesian inference analysis was performed with MrBayes v. 3.1.2 (Huelsenbeck & Ronquist 2001). The GTR + G as nucleotide substitution model was selected using jModeltest v. 0.1.1 (Posada 2008). One million generations with four chains were run and a tree every 100th generation was saved. The first 1000 of the 10,000 saved trees were discarded as burn-in. The resulting 50% majority-rule consensus tree was visualized with TreeViewX (Page 1996). Branch support was calculated as posterior probability (PP).

Taxonomy

Pseudocercosporella stellariicola M.J. Park, J.H. Park & H.D. Shin, **sp. nov.** Fig. 1

MycoBank MB 563164

Similar to *Pseudocercosporella cerastii,* but with wider (2–3 µm) conidia and distinct leaf lesions. Similar to *P. woronovii,* but with shorter (40–80 µm), straight to slightly curved, 1–3(–4)-euseptate conidia.

Type: On living leaves of *Stellaria aquatica* (L.) Scop. (*Caryophyllaceae*), KOREA, Namyangju, Experimental Farm of Korea University, 37°35′01″N 127°14′13″E, 3 May 2006, H.D. Shin & M.J. Park (**Holotype**, KUS-F21740; culture ex-type, KACC42363).

Etymology: The epithet is derived from the host plant genus.

Leaf spots amphigenous, circular to subcircular, 2–7 mm diam., whitish to pale brown. Caespituli amphigenous, grayish-brown, punctiform, evenly and more or less densely distributed over the spot. Mycelium internal; hyphae septate, branched, hyaline, 3–5 µm wide; superficial mycelium absent. Stromata well-developed, erumpent, subcuticular or intraepidermal, sometimes substomatal, consisting of medium brown, pseudoparenchymatal cells, ca. 30–100 µm wide and 25–65 µm high. Conidiophores numerous in a dense divergent fascicle, hyaline throughout, aseptate, straight, conic to

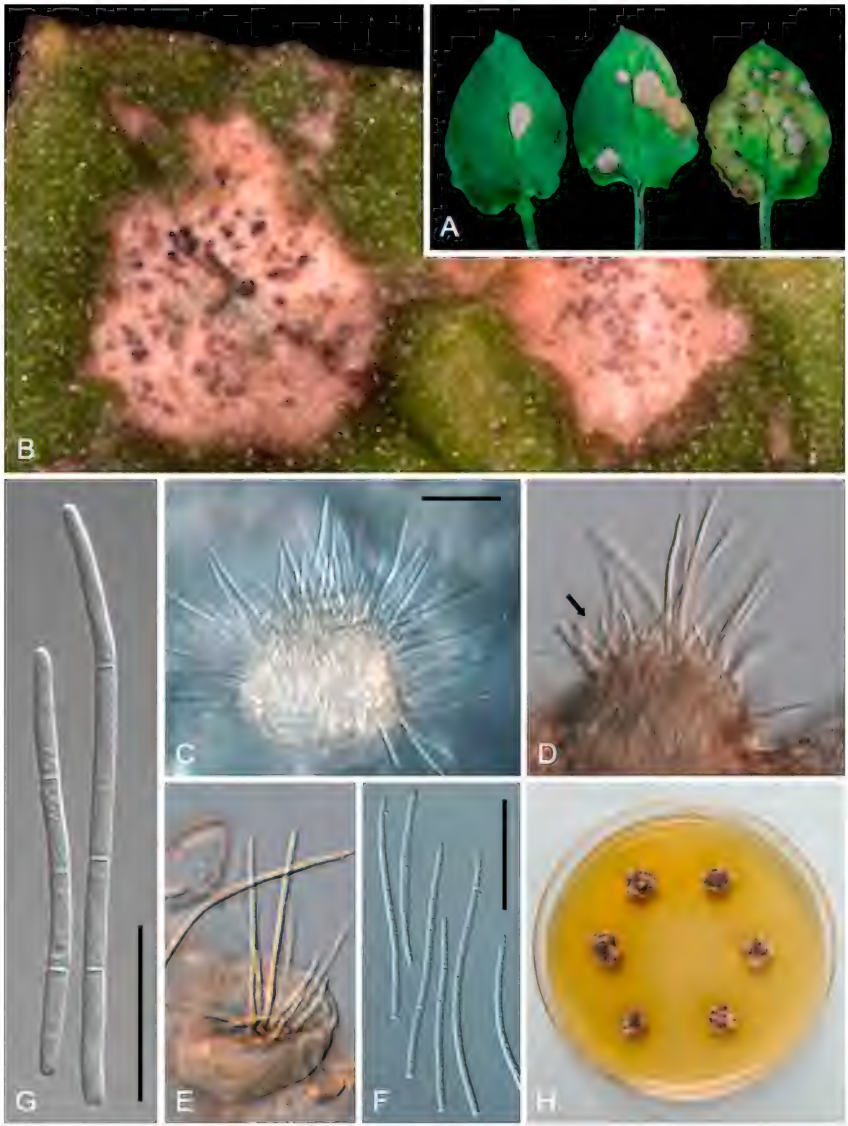

Fig. 1. *Pseudocercosporella stellariicola*. A: Symptoms on leaves of *Stellaria aquatica*. B: Close-up of the leaf lesions showing many stromata on the infected tissue. C: Upper view of a stroma emerging from a stoma of the host plant. D: Vertical view of a stroma protruding from the cuticle of the host plant. E: Small stroma with a few conidiophores emerging through the substomatal cavity. F: Conidia. G: Close-up of conidia. H: Six-week-old colonies formed on potato dextrose agar. Scale bar: C–D = 50 µm, E–F = 30 µm, G = 20 µm.

mildly geniculate-sinuous, 8–15(–20) µm in length, 2–3 µm in width; conidial scars unthickened, not darkened. Conidia solitary, filiform, straight to slightly curved, hyaline, 1–3(–4)-euseptate, non-constricted at the septa, irregularly guttulate, obtuse at the apex, subtruncate to subobtuse at the base, 40–80 × 2–3 µm; hilum unthickened, not darkened. Colonies on PDA attaining ca. 10 mm diam after 6 weeks at 25°C under 12-h fluorescent light, dark brownish to blackish, somewhat crumpled, consisting of a slimy mass due to abundant sporulation, producing sparse aerial mycelia. Teleomorph unknown.

Additional specimens examined – **KOREA**, Yangpyeong, Experimental Forest of Korea University, 37°30′12″N 127°41′55″E, 5 May 2004, H.D. Shin, KUS-F20158; 26 May 2004, H.D. Shin, KUS-F20239 (culture: CPC11297); 20 May 2005, H.D. Shin, KUS-F21102; 25 May 2006, H.D. Shin & M.J. Park, KUS-F21808 (culture: KACC42395); Cheongun-myeon, 37°32′04″N 127°44′48″E, 20 May 2007, H.D. Shin & M.J. Park, KUS-F22591 (culture: KACC42830); Namyangju, Experimental Farm of Korea University, 37°35′01″N 127°14′13″E, 17 May 2004, H.D. Shin, KUS-F20216; 19 April 2008, H.D. Shin & M.J. Park, KUS-F23281 (culture: KACC43763); 25 April 2011, H.D. Shin & J.H. Park, KUS-F25669; Taean, Nam-myeon, 36°41′11″N 126°17′05″E, 17 April 2007, H.D. Shin & M.J. Park, KUS-F22539; Hongcheon, Sangoan-ri, 37°39′25″N 127°49′18″E, 28 April 2007, H.D. Shin & M.J. Park, KUS-F22565 (culture: KACC42827); Seongdong-ri, 37°44′41″N 127°52′01″E, 29 April 2007, H.D. Shin & M.J. Park, KUS-F22578; Hoengseong, Eupha-ri, 37°29′47″N 127°58′52″E, 29 April 2011, H.D. Shin & J.H. Park, KUS-F25676; Seowon-myeon, 37°31′33″N 127°52′29″E, 6 May 2011, H.D. Shin, KUS-F25679.

Discussion

Two cercosporoid hyphomycetes are presently known to occur on *Stellaria* species. *Cercosporella echinulata* Garb., described on *Myosoton aquaticum* (L.) Moench (≡ *Stellaria aquatica*), differs from the new species by conidiophores with an echinulate, denticulate apical part. Additionally, *C. echinulata* possesses much longer (35–110 µm) conidiophores and narrower (1–2 µm) conidia. Although the identity of *C. echinulata* is unclear (according to Braun 1995), it is easily differentiated from *Ps. stellariicola*. *Phacellium episphaerium* (Desm.) U. Braun is also readily differentiated from the present species by its synnematous fascicles of conidiophores and aseptate conidia.

Two *Pseudocercosporella* species are known on other caryophyllaceous host plants. *Pseudocercosporella cerastii* (H.C. Greene) U. Braun (Braun 1995) known on *Cerastium* spp. from Central Asia and North America is distinguished from the new species by much narrower (0.75–2 µm) conidia. The leaf lesions associated with *Ps. cerastii* are almost absent or diffuse, whereas those of *Ps. stellariicola* are quite distinct by being circular to subcircular and delimited by a dark border.

Pseudocercosporella woronovii (Siemaszko) U. Braun recorded on *Silene* spp. and *Lychnis* spp. in Europe (see Braun 1995) also produces (sub-)circular lesions and amphigenous colonies on the leaves. However, *Ps. woronovii* differs

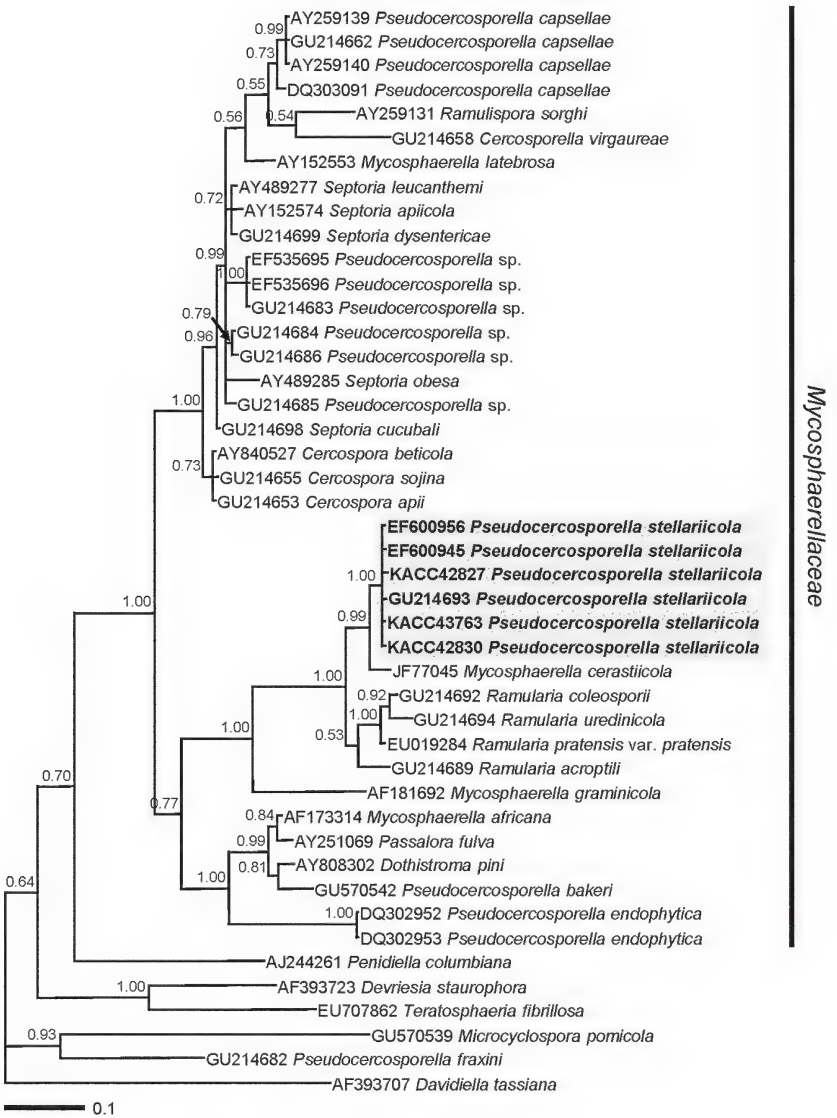

FIG. 2. A 50% majority-rule consensus tree from Bayesian analysis of the ITS rDNA sequences of *Pseudocercosporella* spp. and closely related taxa belonging to *Mycosphaerellaceae*. Numbers above the branches indicate the posterior probability values. The number of nucleotide changes between taxa is represented by branch length and the scale bar corresponds to the number of substitution per site. The sequences of *Ps. stellariicola* are shown in bold and a well-supported clade accommodating them is highlighted in gray box.

from *Ps. stellariicola* by longer (50–100 μm), straight to flexuous, obscurely 1–5-septate conidia.

The complete ITS rDNA sequence data of the six *Ps. stellariicola* isolates obtained were nearly identical, with only one base pair difference between KACC42827 and the other isolates. They formed a separate clade distinct from the other species of *Mycosphaerellaceae* (1.00 PP value) in the Bayesian tree (Fig. 2). Our phylogenetic analysis indicated that *Mycosphaerella cerastiicola* Crous is sister to the *Ps. stellariicola* clade (0.99 PP value); *Ramularia* spp. are also phylogenetically close to the new species. In recent studies, based on 18S, 28S, and ITS-5.8S rDNA sequence analyses (Crous et al. 2009 & 2011), isolate CPC11297 (representing *Ps. stellariicola*) was tentatively identified as a *Ramularia* sp. based on its placement in the *Ramularia* clade. Nevertheless, based on the morphological characteristics described in this study, the isolate is better accommodated in *Pseudocercosporella*. In addition, the fact that *Ps. stellariicola* is closely related with *Ramularia* spp. is not surprising, as *Pseudocercosporella* is polyphyletic within *Mycosphaerellaceae* and *Capnodiales*, as shown in previous phylogenetic studies (Crous et al. 2009, Frank et al. 2010) and the present analysis. In conclusion, both morphological and molecular data support *Ps. stellariicola* as a novel species.

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A key to the peltigericolous fungi in Turkey

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Abstract — Eleven taxa of peltigericolous fungi are reported from Turkey for the first time – *Arthonia fuscopurpurea*, *A. peltigerina*, *Corticifraga fuckelii*, *C. peltigerae*, *Nectriopsis lecanodes*, *Norrlinia peltigericola*, *Polycoccum peltigerae*, *Scutula epiblastematica*, *Stigmidium leucophlebiae*, *Thelocarpon epibolum* var. *epithallinum,* and *Zwackhiomyces kiszkianus*. A key is provided to the 19 specific and infraspecific peltigericolous fungal taxa reported from Turkey. Also provided are additional references, a list of the distributions in Turkey, and a host index.

Key words — *Ascomycota*, lichenicolous fungi, *Peltigera*

Introduction

During our 2008–10 project, "Taxonomical research on *Peltigera* species distributed in western and eastern Black Sea region of Turkey and their lichenicolous fungi," we collected hundreds of *Peltigera* specimens and cited the distribution of *Peltigera* species in about two thirds of Turkey in our final project report (unpublished). The lichenized fungus genus *Peltigera* is a well-known exceptionally rich host for the obligately lichenicolous fungi known as "peltigericolous fungi" (Miądlikowska & Alstrup 1995, Candan et al. 2010). More than 80 species of peltigericolous fungi have been reported worldwide (Hawksworth 1980, Hawksworth & Miądlikowska 1997). Hawksworth (1980) cited the fact that *Peltigera* is one of the richest genera to harbor lichenicolous fungi as evidence that it is derived from a particularly ancient ascomycete group.

Only eight peltigericolous species (Halıcı 2008; Candan et al. 2010) have been included among the 166 lichenicolous fungal species reported from Turkey (Candan & Halıcı 2011, Halıcı & Candan 2011, Kocakaya et al. 2011). Our addition here of 11 peltigericolous taxa raises that total to 19 peltigericolous and 177 lichenicolous fungi now known from the country. We provide a key

to the known peltigericolous fungi with their distribution and host species in Turkey to encourage further study of the group in Turkey.

Material & methods

All materials (except *Refractohilum peltigerae*) of the 19 cited taxa were studied personally and are deposited in ANES (Eskişehir, Turkey). Specimens were examined with a Leica DM 1000 research microscope. Hand sections were examined in K (10% KOH solution), I (Merck Lugol's iodine), and water. Ascospore measurements were made in water. Under 'Taxonomy' we provide as much information as possible from the Turkish specimens studied for taxa new to Turkey and refer to more detailed descriptions for all taxa. A map showing the geographical provinces of Turkey (Fig. 1) and provincial vehicle registration codes (Table 1) summarize the distribution of the taxa in Turkey. A host index (Table 2) is also provided.

Taxonomy

Arthonia fuscopurpurea (Tul.) R. Sant.

A detailed description is provided by Santesson (1960).

The Turkish specimen, collected on a thallus of *Peltigera polydactylon*, seems weakly parasitic, causing bleaching of infected parts of the host thallus. Ascomata arthonioid, sometimes grouped together. Hymenium hyaline, K/I+ blue, I+ red. Epihymenium greenish brown. Asci 8-spored, ascospores hyaline, 1-septate, 9–11 × 3–4 µm. Distribution in Turkey: 29.

Material examined: **TURKEY. Gümüşhane**, Torul, southern slopes of Zigana Mountain, 40°38′28″N 39°23′50″E, alt. 2100 m, on *Peltigera polydactylon*, 04.09.2009, M. Candan, M.G. Halıcı & O. Sezer (ANES 14199).

Arthonia peltigerina (Almq.) H. Olivier

A detailed description is provided by Almquist (1880).

Turkish specimens, collected on thalli of *Peltigera canina* and *P. praetextata*, seem parasitic, bleaching the infected parts of the host thalli. Infection is generally seen in the old parts of the host thalli. Ascomata arthonioid, < 0.5 mm diam., often grouped together in subconcentric rings, sometimes grouped together. Hymenium hyaline, K/I+ blue, I+ red, dextrinoid. Epihymenium brown, slightly greenish tones available, I+ reddish brown. Asci 8-spored, I+ yellowish orange. Ascospores hyaline, 1-septate, cells with oil droplets, with a gelatinous sheath, 14–17 × 5–7 µm. Distribution in Turkey: 03.

Material examined: **TURKEY. Afyon**, Sultandağı, Sultandağı Mountains, 38°28′35″N 31°10′48″E, alt. 1622 m, in *Quercus vulcanica* forest, on *Peltigera canina*, 11.05.2010, M. Candan (ANES 13824); 38°28′48″N 31°11′12″E, alt. 1692 m, in *Quercus vulcanica* forest, on *Peltigera praetextata* on mosses, 11.05.2010, M. Candan (ANES 13836).

Capronia peltigerae (Fuckel) D.Hawksw.

Detailed descriptions of the species are provided by Hawksworth (1980) and Zhurbenko (2004) and of the Turkish specimens by Candan & al. (2010).

Specimens were collected on thalli of *Peltigera canina*, *P. leucophlebia*, *P. monticola* and *P. neckeri*. Distribution in Turkey: 03, 09, 26, 29, 35, 37, 48.

Material examined: **TURKEY. Aydın**, Nazilli, north of Kavaklıdere, 37°27′39″N 28°20′39″E, alt. 800 m, in *Pinus brutia* forest, on *Peltigera neckeri*, 15.06.2009, M. Candan & O. Sezer (ANES 14220); **Eskişehir**, İnönü, south of Erenköy Village, 39°44′N 30°01′E, alt. 1100 m, in *Quercus* sp. communities and *Pinus nigra* forest, on *Peltigera monticola*, 02.05.2010, M. Candan (ANES 14216); **Gümüşhane**, Torul, exit of Torul, southern slopes of Zigana pass, 40°38′04″N 39°23′12″E, alt. 1019 m, in *Picea* and *Abies* forest, on *Peltigera canina*, 04.09.2009, M. Candan & M.G. Halıcı (ANES 14217); **İzmir**, Kemalpaşa, Nif Mountain, 38°24′04″N 27°23′52″E, alt. 869 m, in *Pinus nigra* forest, on *Peltigera neckeri*, 16.06.2009, M. Candan & O. Sezer (ANES 14218); **Kastamonu**, Şenpazar, southwest of Sada Village, Küre Mountains, 41°43′45″N 33°29′04″E, alt. 830 m, in *Abies nordmanniana* and *Pinus sylvestris* mixed forest, on *Peltigera leucophlebia*, 30.07.2010, M. Candan & M.G. Halıcı (ANES 14219).

Corticifraga fuckelii (Rehm) D. Hawksw. & R. Sant.

A detailed description is provided by Hawksworth & Santesson (1990).

The Turkish specimen, collected on thalli of *Peltigera* sp. (with gyrophoric acid determined by TLC), seems parasymbiotic (no visible damage to the host thallus). Ascomata apothecia, 0.2–0.3 mm diam., not arising in circular spots of the host thallus. Interascal filaments with a hyaline end cell, 1.5–2 µm diam. Hymenium 50–60 µm tall. Asci 8-spored, I+ red. Ascospores hyaline, 1–2-septate, 15–18 × 5–7 µm. Distribution in Turkey: 08.

Material examined: **TURKEY. Artvin**, Şavşat, northeast of Şavşat, 41°13′67″N 42°22′58″E, alt. 1360 m, in *Picea orientalis* forest, on *Peltigera* sp. (with gyrophoric acid) on mosses, 31.08.2009, M. Candan, M.G. Halıcı & Okan Sezer (ANES 13825).

Corticifraga peltigerae (Fuckel) D. Hawksw. & R. Sant.

A detailed description is provided by Hawksworth & Santesson (1990).

The Turkish specimens, collected on thalli of *Peltigera canina*, *P. monticola* and *P. rufescens*, seem parasitic (bleaching seen in the infected parts of the host thalli). Ascomata apothecia, 0.2–0.3 mm diam., arising in circular spots of the host thallus. Interascal filaments with a brown end cell (not a good taxonomical character, in our opinion, as this is indistinct in sections from young ascomata). Hymenium 50–60 µm tall. Asci 8-spored. Ascospores hyaline, 1–3-septate, 12–20 × 5–7 µm. Distribution in Turkey: 08, 42, 45, 66.

Material examined: **TURKEY. Artvin**, Şavşat, northeast of Şavşat, 41°13′67″N 42°22′58″E, alt. 1360 m, in *Picea orientalis* forest, on *Peltigera rufescens*, 31.08.2009, M. Candan, M.G. Halıcı & O. Sezer (ANES 13830); Ardanuç, 41°13′52″N 42°05′31″E, alt. 472 m, in *Alnus-Quercus* forest, on *Peltigera canina*, 31.08.2009; M. Candan, M.G. Halıcı & O. Sezer (ANES14221); **Manisa**, Akhisar, Sındırgı road, east of İsaca Village, 39°09′35″N 28°01′29″E, alt. 490 m, in *Pinus brutia* forest, on *Peltigera monticola* , 17.06.2009; M. Candan & O. Sezer (ANES 13829); **Konya**, Bozkır-Belören, west of Aydınkışla Village, 37°13′21″N 32°21′27″E, alt. 1400 m, in *Juniperus* sp. communities, on *Peltigera monticola*, 05.08.2009, M. Candan & M.G. Halıcı (ANES 13826); **Yozgat**, 10 km to Yozgat, 39°37′13″N 34°56′53″E, alt. 1015 m, on *Peltigera rufescens*, 25.08.2009. M. Candan, M.G. Halıcı & O. Sezer (ANES 13828).

Dacampia rufescentis (Vouaux) D. Hawksw.

Detailed descriptions of the species are provided by Vouaux (1912) and Hawksworth (1986) and of the Turkish specimen by Candan & al. (2010).

Specimens were collected on thalli of *Peltigera kristinssonii*, *P. ponojensis*, *P. praetextata,* and *P. rufescens.* Distribution in Turkey: 26, 32, 37, 66.

Material examined: **TURKEY. Isparta**, Eğirdir, east of Mahmatlar Village, northern slopes of Eğirdir Lake, 37°57′11″N 30°56′41″E, alt. 940 m, in siliceous rocks, on *Peltigera kristinssonii*, 31.07.2009, M. Candan & M.G. Halıcı (ANES 14213). **Kastamonu,** Seydiler, southeast of Seydiler, Kastamonu highway, Oyrak passage, 41°34′26″N 33°46′43″E, alt. 1200 m, in *Salix* sp., *Populus* sp., and open areas, on *Peltigera rufescens*, 30.07.2010, M. Candan & M.G. Halıcı (ANES 14214); **Yozgat,** Çayıralan, Çat forests, 39°19′30″N 35°50′17″E, alt. 1650 m, in *Pinus sylvestris* forest, on *Peltigera ponojensis*, 27.08.2009, M. Candan, M.G. Halıcı & O. Sezer (ANES 14215).

Nectriopsis lecanodes (Ces.) Diederich & Schroers

Detailed descriptions are provided by Booth (1959) and Sérusiaux et al. (1999).

Turkish specimens, collected on thalli of *Peltigera leucophlebia,* seem parasitic (extensive damage seen in the infected parts of the host thalli). Ascomata perithecioid, 150–300 µm diam., pinkish to orange, often covered with a whitish tomentum, arising on a small stalk. Hymenium orange. Asci 8-spored. Ascospores oblique, uniseriately arranged in the asci, hyaline, 1-septate, one or two oil droplets present in each cell, ellipsoid, round in the apices, more or less verruculose, 10–12 × 4–5 µm. Distribution in Turkey: 25, 37.

Material examined: **TURKEY. Erzurum,** Olur, southeast of Yukarıkaracasu Village, 40°49′26″N 42°17′11″E, alt. 1800 m, in *Pinus sylvestris* forest, on *Peltigera leucophlebia* on mosses, 30.08.2009, M. Candan, M.G. Halıcı & O. Sezer (ANES 14203); **Kastamonu,** Azdavay, southwest of Sada Village, Küre Mountains, 41°43′45″N 33°29′04″E, alt. 830 m, in *Abies nordmanniana* and *Pinus sylvestris* forest, on *Peltigera leucophlebia* on mosses, 30.07.2010, M. Candan & M.G. Halıcı (ANES 13837).

Neolamya peltigerae (Mont.) Theiss. & Syd.

Detailed descriptions of the species are provided by Keissler (1930) and Ertz et al. (2004) and of the Turkish specimen by Candan & al. (2010).

Specimens were collected on thalli of *Peltigera didactyla.* Distribution in Turkey: 08, 55.

Material examined: **TURKEY. Samsun**, Ayvacık, northwest of Hasan Uğurlu Dam Lake, 40°56′33″N 36°38′21″E, alt. 80 m, in siliceous rocks, on *Peltigera didactyla*, 07.09.2009, M. Candan, M.G. Halıcı & O. Sezer (ANES 14202).

Norrlinia peltigericola (Nyl.) Theiss. & Syd.

Detailed descriptions are provided by Hawksworth (1980) and Martinez & Hafellner (1998).

The Turkish specimen, collected on a *Peltigera praetextata* thallus, seems parasymbiotic (no visible damage to the host lichen). Ascomata perithecioid,

black, aggregated in small groups, immersed only at the base, wall blackish in the outer part, brown in the inner part. Interascal filaments not present, oil droplets present. Asci 2-spored. Ascospores hyaline, rarely very pale brownish, muriform with 12 transsepta and 4 longisepta, 42–52 × 14–28 µm. Distribution in Turkey: 09.

Material examined: **TURKEY. Aydın,** Bozdağan, Madran Mountain, south of Altıntaş Village, 37°35′40″N 28°21′00″E, alt. 800 m, in *Pinus brutia* forest, on *Peltigera praetextata*, 15.06.2009, M. Candan & O. Sezer (ANES 13833).

Phoma peltigerae (P. Karst.) D. Hawksw.

Detailed descriptions of the species are provided by Keissler (1930) and Hawksworth (1981) and of the Turkish specimens by Candan & al. (2010).

Specimens were collected on thalli of *Peltigera canina*, *P. elisabethae,* and *P. rufescens.* Distribution in Turkey: 11, 16, 43.

Polycoccum crassum Vězda

A detailed description of the species is provided by Hawksworth & Diederich (1988) and of the Turkish specimens by Halıcı et al. (2007) and Candan & al. (2010).

Specimens were collected on thalli of *Peltigera canina*, *P. elisabethae*, *P. monticola,* and *P. neckeri.* Distribution in Turkey: 08, 29, 35, 44, 48, 66.

Material examined: **TURKEY. Artvin,** Şavşat, northeast of Şavşat, 41°13′67″N 42°22′58″E, alt. 1360 m, in *Picea orientalis* forest, on *Peltigera elisabethae*, 31.08.2009, M. Candan, M.G. Halıcı & O. Sezer (ANES 14206); **Gümüşhane,** Torul, exit of Torul, southern slopes of Zigana pass, 40°38′04″N 39°23′12″E, alt. 1019 m, in *Picea* and *Abies* forest, on *Peltigera neckeri*, 04.09.2009, M. Candan, M.G. Halıcı & O. Sezer (ANES 14207); **İzmir,** Kemalpaşa, Nif Mountain, 38°24′50″N 27°23′40″E, alt. 710 m, in *Pinus brutia* forest, on *Peltigera neckeri,* 16.06.2009, M. Candan & O. Sezer (ANES 14208); **Yozgat**, Çayıralan, Çat forest enterance, Sivas province border, 39°19′30″N 35°50′17″E, alt. 1650 m, in *Pinus sylvestris* forest, on *Peltigera monticola*, 27.08.2009, M. Candan, M. G. Halıcı & O. Sezer (ANES 14209).

Polycoccum peltigerae (Fuckel) Vězda

A detailed description is provided by Hawksworth & Diederich (1988).

Turkish specimens, collected on thalli of *Peltigera leucophlebia* and *P. rufescens,* seem slightly parasitic (swellings seen on the host lichens; heavy infections may kill parts of the host thallus). Ascomata perithecioid, ~ 200 µm diam., entirely immersed in the host lichens, becoming erumpent only in the ostiolar region. Asci 8-spored. Ascospores brown, 1-septate, ellipsoid to fusiform, cells generally attenuated at the ends, 14–18 × 5–6 µm. Distribution in Turkey: 08, 37.

Material examined: **TURKEY. Artvin,** Şavşat, northeast of Şavşat, 41°13′67″N 42°22′58″E, alt. 1360 m, in *Picea orientalis* forest, on *Peltigera rufescens*, 31.08.2009, M. Candan, M. G. Halıcı & O. Sezer (ANES 14204); **Kastamonu,** near Azdavay, southwest of Sada Village, Küre Mountains, alt. 830 m, in *Abies nordmanniana* and *Pinus sylvestris* forest, on *Peltigera leucophlebia* , 30.07.2010. M. Candan & M.G. Halıcı (ANES 14205).

Pronectria robergei (Mont. & Desm.) Lowen

A detailed description of the species is provided by Keissler (1930) and of the Turkish specimen by Candan & al. (2010).

The specimen was collected on thallus of *Peltigera rufescens*. Distribution in Turkey: 08.

Refractohilum peltigerae (Keissl.) D. Hawksw.

Detailed descriptions of the species are provided by Hawksworth (1977, 1979) and of the Turkish specimen by John & Breuss (2004) and Halıcı (2008).

The specimen was collected on thallus of *Peltigera polydactylon*. Distribution in Turkey: 61.

Scutula epiblastematica (Wallr.) Rehm

Detailed descriptions are provided by Triebel et al. (1997) and Clauzade et al. (1989).

The Turkish specimen, collected on thallus of *Peltigera praetextata*, seems parasitic (host algal photobiont depleted in the infected parts of the thallus). Ascomata apothecioid, often aggregated, cream coloured when young, becoming greenish black when mature. Inner part of exciple purplish brown Hymenium hyaline, I+ red, hypothecium pale brownish. Asci *Scutula*-type (with a fuzzy K/I+ blue tube-like structure in the tholus). Ascospores hyaline, 1-septate, with oil droplets, 8–11 × 3–4 µm. Distribution in Turkey: 67.

Material examined: **TURKEY. Zonguldak**, Devrek, north of İsabeyli Village, Babadağ Mountain, 41°13′23″N 31°52′57″E, alt. 600 m, in *Carpinus* sp. and *Fagus orientalis* forest, on *Peltigera praetextata* on mosses, 29.07.2010, M. Candan & M.G. Halıcı (ANES 14198).

Stigmidium leucophlebiae Cl. Roux & Triebel

A detailed description is provided by Roux & Triebel (1994).

Turkish specimens, collected on thalli of *Peltigera leucophlebia*, seem parasitic (infected host thallus parts brownish or blackish). Ascomata perithecioid, 50–60 µm diam., semi-immersed. Vegetative hyphae sometimes visible on the host thalli. Asci 8-spored. Ascospores hyaline, 1-septate, cells not equal in size, with a gelatinous sheath, 9–14 × 4–5 µm. Distribution in Turkey: 37, 78.

Material examined: **TURKEY. Karabük**, Yenice, Şeker canyon, 41°11′26″N 32°21′35″E, alt. 230 m, in mixed *Pinus nigra* and broad leaved tree forest, on *Peltigera leucophlebia*, 01.06.2009, A. Türk & M. Candan (ANES 13834); **Kastamonu**, Şenpazar, southwest of Sada Village, Küre Mountains, 41°43′45″N 33°29′04″E, alt. 830 m, in *Abies nordmanniana* and *Pinus sylvestris* mixed forest, on *Peltigera leucophlebia*, 30.07.2010, M. Candan & M. G. Halıcı (ANES 13835).

Stigmidium peltideae (Vain.) R.Sant.

A detailed description of the species is provided by Roux & Triebel (1994) and of the Turkish specimens by Candan & al. (2010).

Specimens were collected on thalli of *Peltigera collina*, *P. neckeri*, and *P. praetextata*. Distribution in Turkey: 03, 09, 26, 29, 48, 66.

Material examined: **TURKEY. Aydın**, Nazilli, Semailli Village road, 38°01′12″N 28°18′24″E, alt. 850 m, in *Quercus* forest and siliceous rocks, on *Peltigera neckeri*, 15.06.2009, M. Candan & O. Sezer (ANES 14210); **Eskişehir**, Türkmen Dağı, Gölcük road, 39°29′N 30°21′E, alt. 1320 m, in siliceous rocks, on *Peltigera collina*, 24.03.2009, M. Candan (ANES 14211); **Gümüşhane**, Torul, exit of Torul, southern slopes of Zigana pass, 40°38′04″N 39°23′12″E, alt. 1019 m, in *Picea* and *Abies* forest, on *Peltigera neckeri*, 04.09.2009, M. Candan, M.G. Halıcı & O. Sezer (ANES 14212); **Muğla**, Yatağan, Yatağan old road, Gökbel pass, 37°25′34″N 28°08′52″E, alt. 323 m, on *Peltigera neckeri*, 15.06.2009, M. Candan & O. Sezer (ANES 14222); **Yozgat**, Akdağmadeni, east of Akdağmadeni, 39°40′00″N 35°56′07″E, alt. 1603 m, in *Pinus sylvestris* forest, on *Peltigera neckeri*, 26.08.2009, M. Candan, M.G. Halıcı & O. Sezer (ANES 14223).

Thelocarpon epibolum* var. *epithallinum (Leight. ex Nyl.) G. Salisb.

Detailed descriptions are provided by Salisbury (1966) and Ahti (1973).

The Turkish specimen, collected on a *Peltigera leucophlebia* thallus, seems parasymbiotic (no visible damage to the host lichen seen). Ascomata perithecioid, superficial, bright yellow, citrine green when fresh, pruinose, 150–250 µm diam. Interascal filaments present, hyaline, oil droplets present. Asci more than 50-spored. Ascospores hyaline, simple, 9–11 × 3–4 µm. Distribution in Turkey: 78.

Material examined: **TURKEY. Karabük**, Şeker, Yenice, Şeker canyon, 41°11′26″N 32°21′35″E, alt. 230 m, in mixed *Pinus nigra* and broad leaved tree forest, on *Peltigera leucophlebia*, 01.06.2009, A. Türk & M. Candan (ANES 13831).

Zwackhiomyces kiszkianus D. Hawksw. & Miądl.

A detailed description is provided by Hawksworth & Miądlikowska (1997).

The Turkish specimen, collected on a *Peltigera elisabethae* thallus, seems parasymbiotic (no visible damage to the host lichen seen). Ascomata perithecioid, scattered, immersed, exposed only at the ostiole. Asci (6–)8-spored. Ascospores hyaline, ellipsoid to somewhat soleiform, 1-septate, constricted at the septum, 25–27 × 11–12 µm. Distribution in Turkey: 08.

Material examined: **TURKEY. Artvin**, Şavşat, northeast of Şavşat, 41°13′67″N 42°22′58″E, alt. 1360 m, in *Picea orientalis* forest, on *Peltigera elisabethae*, 31.08.2009, M. Candan, M.G. Halıcı & O. Sezer (ANES 14201).

Key to the peltigericolous fungi species in Turkey

1. Spores produced in asci 2
1. Spores not produced in asci **18**

2(1). Ascomata perithecioid 3
2. Ascomata arthonioid or apothecioid **14**

3(2). Ascospores brown when mature 4
3. Ascomata hyaline or rarely very pale brown when mature 7

4(3). Ascospores 1-septate 5
4. Ascospores 3-septate or muriform with trans- and longisepta 6

5(3). Perithecia 300–500 µm diam., in general on the lower surface of the host lichen. Asci 4-spored. Ascospores 24–30 × 8–10 µm ..... ***Polycoccum crassum***
5. Perithecia ~ 200 µm diam., entirely immersed in the host lichen, becoming erumpent only in the ostiolar region. Asci 8-spored. Ascospores 14–18 × 5–6 µm ..... ***Polycoccum peltigerae***

6(4). Perithecia covered with stiff, smooth-walled, and dark brown hairs ..... ***Capronia peltigerae***
6. Perithecia without hairs. ..... ***Dacampia rufescentis***

7(3). Asci containing more than 8 spores 8
7. Asci up to 8-spored 9

8(5). Perithecia immersed to slightly erumpent, black. Asci 16-spored ..... ***Neolamya peltigerae***
8. Perithecia superficial, bright yellow, citrine green when fresh, pruinose. Asci more than 50-spored ..... ***Thelocarpon epibolum* var. *epithallinum***

9(7). Asci 8-spored **10**
9. Asci 2-spored. ..... ***Norrlinia peltigericola***

10(9). Perithecia in pink, orange or reddish tones **11**
10. Perithecia black **12**

11(10). Perithecia pinkish to orange, often covered with a whitish tomentum, arising on a small stalk ..... ***Nectriopsis lecanodes***
11. Perithecia orange-red, becoming yellowish when old; immersed to semi-immersed ..... ***Pronectria robergei***

12(10). Interascal filaments absent **13**
12. Interascal filaments present ..... ***Zwackhiomyces kiszkianus***

13(12). Perithecia 50–60 µm diam. Ascospores 9–14 × 4–5 µm ..... ***Stigmidium leucophlebiae***
13. Perithecia 35–40 µm diam. Ascospores 11–12 × 2.5–3 µm ..... ***Stigmidium peltideae***

14(2). Ascomata apothecioid **15**
14. Ascomata arthonioid **17**

15(14). Asci not *Scutula*-type **15**
15. Asci *Scutula*-type. ..... ***Scutula epiblastematica***

16(15). Ascomata not arising in circular spots of the host thallus, not bleaching the thallus. Interascal filaments with a hyaline end cell, 1.5–2 µm diam ..... ***Corticifraga fuckelii***
16. Ascomata arising in circular bleached spots of the host thallus. Interascal filaments with a brown end cell ..... ***Corticifraga peltigerae***

17(14). Ascomata often grouped together in subconcentric rings. Ascospores 14–17 × 5–7 µm ..... ***Arthonia peltigerina***

17. Ascomata sometimes grouped together. Ascospores 9–11 × 3–4 µm ..... ***Arthonia fuscopurpurea***

18(1). Conidia produced within pycnidia (coelomycetes). ..... ***Phoma peltigerae***

18. Conidia not produced within pycnidia (hyphomycetes) ..... ***Refractohilum peltigerae***

Acknowledgements

Kerry Knudsen (UCR) and Wolfgang von Brackel (Germany) are thanked for reviewing this paper. Shaun Pennycook is also thanked for his very useful comments on formatting the manuscript. This study was supported by TUBITAK (108T556 coded project).

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Table 1. Provincial vehicle registration codes.

Code	Province	Code	Province
01	Adana	42	Konya
02	Adıyaman	43	Kütahya
03	Afyon	44	Malatya
04	Ağrı	45	Manisa
05	Amasya	46	Kahramanmaraş
06	Ankara	47	Mardin
07	Antalya	48	Muğla
08	Artvin	49	Muş
09	Aydın	50	Nevşehir
10	Balıkesir	51	Niğde
11	Bilecik	52	Ordu
12	Bingöl	53	Rize
13	Bitlis	54	Sakarya
14	Bolu	55	Samsun
15	Burdur	56	Siirt
16	Bursa	57	Sinop
17	Çanakkale	58	Sivas
18	Çankırı	59	Tekirdağ
19	Çorum	60	Tokat
20	Denizli	61	Trabzon
21	Diyarbakır	62	Tunceli
22	Edirne	63	Şanlıurfa
23	Elazığ	64	Uşak
24	Erzincan	65	Van
25	Erzurum	66	Yozgat
26	Eskişehir	67	Zonguldak
27	Gaziantep	68	Aksaray
28	Giresun	69	Bayburt
29	Gümüşhane	70	Karaman
30	Hakkari	71	Kırıkkale
31	Hatay	72	Batman
32	Isparta	73	Şırnak
33	İçel	74	Bartın
34	İstanbul	75	Ardahan
35	İzmir	76	Iğdır
36	Kars	77	Yalova
37	Kastamonu	78	Karabük
38	Kayseri	79	Kilis
39	Kırklareli	80	Osmaniye
40	Kırşehir	81	Düzce
41	Kocaeli		

Table 2. Host index.

HOST LICHEN	LICHENICOLOUS FUNGUS
Peltigera canina	*Arthonia peltigerina*
	Capronia peltigerae
	Corticifraga peltigerae
	Phoma peltigerae
	Polycoccum crassum
Peltigera collina	*Stigmidium peltideae*
Peltigera didactyla	*Neolamya peltigerae*
Peltigera elisabethae	*Phoma peltigerae*
	Polycoccum crassum
	Zwackhiomyces kiszkianus
Peltigera kristinssonii	*Dacampia rufescentis*
Peltigera leucophlebia	*Capronia peltigerae*
	Nectriopsis lecanodes
	Polycoccum peltigerae
	Stigmidium leucophlebiae
	Thelocarpon epibolum var. *epithallinum*
Peltigera monticola	*Capronia peltigerae*
	Corticifraga peltigerae
	Polycoccum crassum
Peltigera neckeri	*Capronia peltigerae*
	Polycoccum crassum
	Stigmidium peltideae
Peltigera polydactylon	*Arthonia fuscopurpurea*
	Refractohilum peltigerae
Peltigera ponojensis	*Dacampia rufescentis*
Peltigera praetextata	*Arthonia peltigerina*
	Dacampia rufescentis
	Norrlinia peltigericola
	Scutula epiblastematica
	Stigmidium peltideae
Peltigera rufescens	*Corticifraga peltigerae*
	Dacampia rufescentis
	Phoma peltigerae
	Polycoccum peltigerae
	Pronectria robergei
Peltigera sp. (with gyrophoric acid)	*Corticifraga fuckelii*

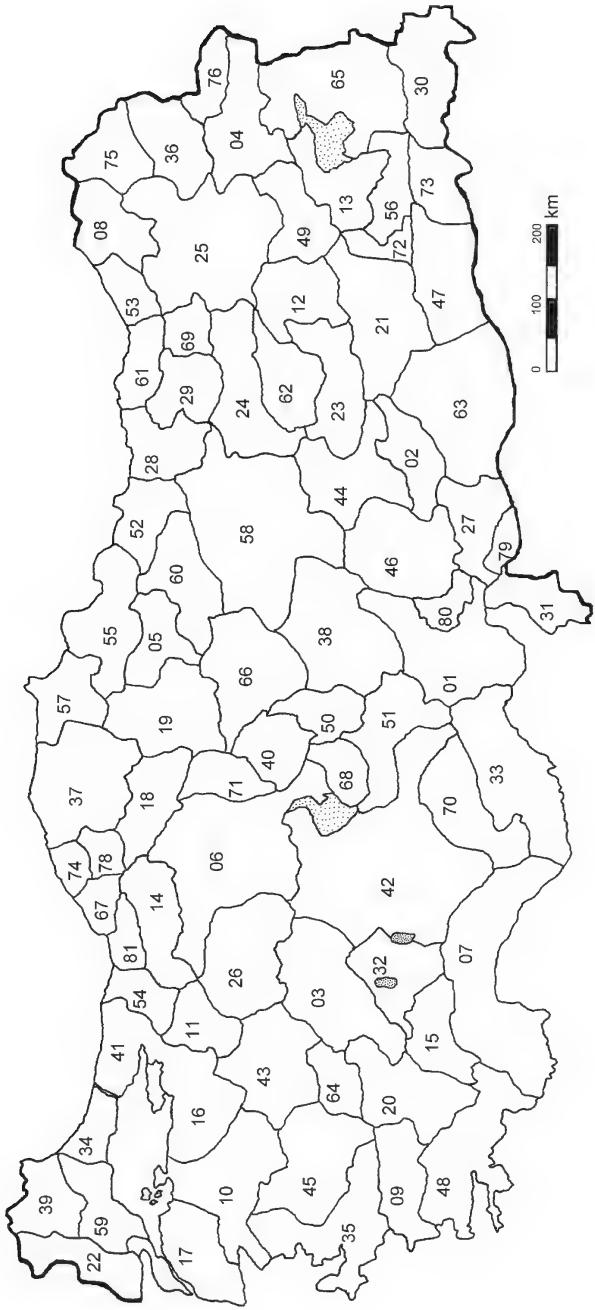

Fig. 1. Turkish provinces and their vehicle registration codes.

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Taxonomy of the *Gymnopus inusitatus* group and the new *G. inusitatus* var. *cystidiatus* from Hungary

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Abstract — The authors describe a new bisporic basidiomycete, *Gymnopus inusitatus* var. *cystidiatus,* collected in Hungary. It differs morphologically from var. *inusitatus* by the presence of well-developed cheilocystidia and ecologically by growing in the sandy grassland under *Salix*. Its taxonomic position is confirmed by DNA studies.

Key words — *Agaricomycetes*, *Agaricales*, *Omphalotaceae,* phylogeny, ecology

Introduction

During his mycological field research in Hungary, the second author found a fungus macroscopically similar to *Mycetinis scorodonius* (Fr.) A.W. Wilson & Desjardin, but lacking a garlic-like smell. Moreover, microscopic studies showed that it belongs to the genus *Gymnopus* (Pers.) Roussel, species group of *G. inusitatus/bisporus/catalonicus* of sect. *Levipedes* (Fr.) Halling, subsect. *Alkalivirentes* Antonín & Noordel. Species of sect. *Levipedes* are characterized by a pileipellis composed of inflated, often lobed to coralloid, elements (dryophila-type) and the taxa of subsect. *Alkalivirentes* by wall encrustations that turn green in alkali (Antonín & Noordeloos 2010), a division supported by molecular studies. DNA sequences of nuclear ITS and partial LSU ribosomal RNA genes from holotypes of the above-mentioned taxa were identical. Based on this result and morphological similarities, we propose synonymy of *G. inusitatus, G. bisporus,* and *G. catalonicus*. We propose a new variety for the Hungarian collection, which differs microscopically and ecologically from the typical *G. inusitatus*.

Materials & methods

Macroscopic description of collected specimens is based on a photo and dry specimens. Colour abbreviations follow Kornerup & Wanscher (1983); herbarium abbreviations follow Holmgren & Holmgren (1998). Nomenclatural authorities are cited according to the International Plant Names Index Authors website (http://www.ipni.org/ipni/authorsearchpage.do). Microscopic features are described from dried material mounted in H_2O, c. 5% KOH, Melzer's reagent, and Congo red using an Olympus BX-50 light microscope with a magnification of 1000×. Measurements are designated for basidiospores by E (quotient of length and width in any one spore) and Q (mean of E-values) and for lamellae by L (number of entire lamellae) and l (number of series of lamellulae between each pair of entire lamellae).

The nuclear ribosomal RNA ITS and LSU regions from the specimen were sequenced as well as those from the types the type specimens of *Gymnopus bisporus* (BCN–SCM B-4065), *G. catalonicus* (BCN–SCM B-4057), and *G. inusitatus* (BCN–SCM B-4058). Both DNA extraction and PCR amplification were conducted twice, with new PCR reagents used each time.

DNA was isolated from dried herbarium specimens using PowerSoil™ DNA Isolation Kit (Mo-Bio). PCR amplifications were performed in the Mastercycler® ep thermocycler (Eppendorf) according to Tomšovský & Jankovský (2008). *Macrogen* Inc. (Seoul, Korea) sequenced the amplified fragments.

Newly obtained sequences were edited manually using BioEdit version 7.0.9. (T. Hall, Ibis Biosciences). Selected sequences from *Gymnopus* sect. *Levipedes* species. published by Mata et al. (2006) were retrieved from GenBank and added to the dataset (Fig. 1). *Gymnopus brassicolens* and *G. impudicus* were selected as outgroup taxa. The ITS alignment with gaps consisted of 790 characters including 630 constant and 160 variable sites.

Bayesian analysis used MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) and best-fit model (GTR+G) and parameters given by MrModeltest (Nylander 2004). Markov chains initiated from a random tree were run for 2,000.000 generations with samples taken every 100th generation. The generation number excluded a burn-in of 200 000 generations; Tracer v1.4.1 (http://tree.bio.ed.ac.uk/software/tracer/) determined the statistics.

The additional PHYML analysis for estimating maximum likelihood phylogenies was run at the server Phylogeny.fr (Dereeper et al. 2008) in "A la Carte" mode. Alignments were treated with Gblock to eliminate poorly aligned positions and ambiguous regions; GTR substitution model was selected for the ITS dataset. Bootstrap branch support values (BP) were estimated in PHYML under the maximum likelihood criterion using default 100 replicates. There were 659 (89% of the original 790 positions) positions analysed in PHYML after Gblock treatment.

The LSU sequences were not analysed due to the limited number of similar sequences available in the Genbank.

Results

Both ITS and LSU sequence analyses showed 100% similarity among the Hungarian collection and the holotypes of *Collybia inusitata*, *C. bispora*, and

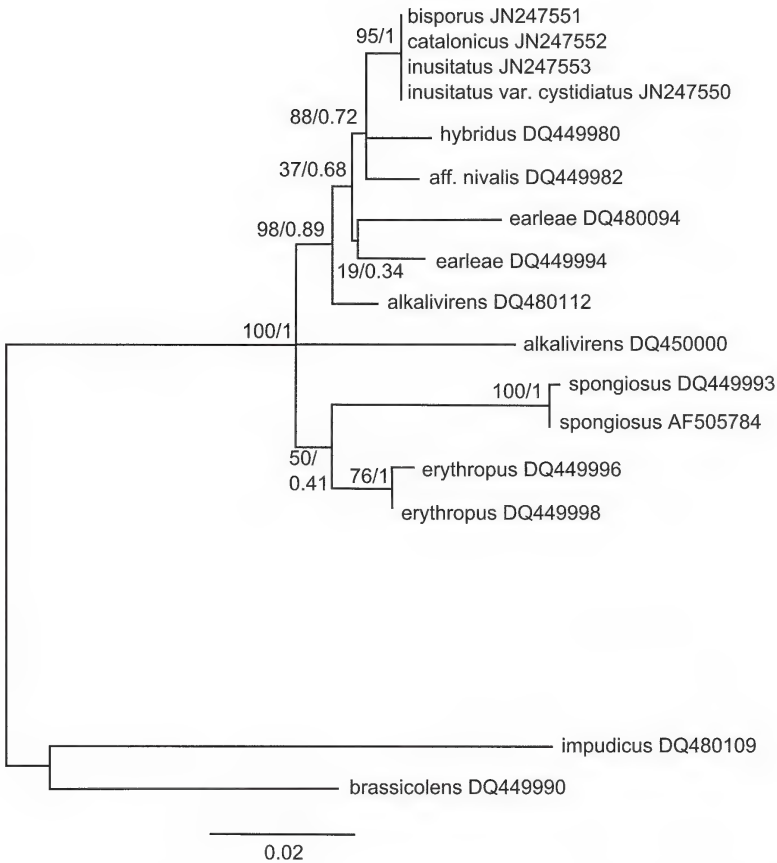

Fig. 1. Bayesian phylogram obtained from the ITS rDNA sequences of *Gymnopus inusitatus* and other taxa of *Gymnopus* sect. *Levipedes*. *Gymnopus brassicolens* and *G. impudicus* were selected as outgroup. Bootstrap support values of Maximum likelihood analysis (Phyml) and Bayesian posterior probability values are given at branches. The bar indicates number of substitutions per nucleotide position.

C. catalonica (Fig. 1), indicating that they are conspecific. Both phylogenetic analyses produced almost identical tree topologies. The Bayesian tree had a likelihood value -2192.343. Empirical base frequencies were pi(A) = 0.247, pi(C) = 0.167, pi(G) = 0.210, and pi(T) = 0.377. Model information: parameter $\alpha = 0.235$; substitution rates A–C = 0.062, A–G = 0,495, A–T = 0.070, C–G = 0.012, C–T = 0.286, G–T = 0.073; tree-length = 0.488.

Fig. 2. *Gymnopus inusitatus* var. *cystidiatus* (holotype). Scale bar = 10 mm. Photo P. Finy.

Taxonomy

Microscopical examination of the types and Hungarian collection noted above confirmed the synonymy indicated by the sequence analyses. We propose the following taxonomy for this species group of bisporic taxa:

Gymnopus inusitatus (Vila & Llimona) Vila & Llimona, Rev. Catal. Micol. 28: 180. 2006.

≡ *Collybia inusitata* Vila & Llimona, Rev. Catal. Micol. 24: 84. 2002.

= *Collybia bispora* J. Carbó & Pérez-De-Greg., Rev. Catal. Micol. 24: 278. 2002.

≡ *Gymnopus bisporus* (J. Carbó & Pérez-De-Greg.) Vila & Llimona, Rev. Catal. Micol. 28: 180. 2006.

= *Collybia catalonica* Vila & Llimona, Rev. Catal. Micol. 24: 80. 2002.

≡ *Gymnopus catalonicus* (Vila & Llimona) Vila & Llimona, Rev. Catal. Micol. 28: 180. 2006.

Gymnopus inusitatus* var. *cystidiatus Antonín, Finy & Tomšovský, **var. nov.**

MycoBank MB 563276 Figs 2–3

A varietate typica cheilocystidiis presentibus et oecologiae (sub Salice*) differt.*

Type: Hungary, Székesfehervár–Sóstó, 14.IX.2010, leg. P. Finy (**Holotype**, BRNM 737257; GenBank JN247550, JN247554).

Etymology: having well-developed cheilocystidia.

Basidiomes similar to *Mycetinis scorodonius*. Pileus up to 10 mm broad, convex to applanate, with plane to depressed centre, smooth, slightly crenulate

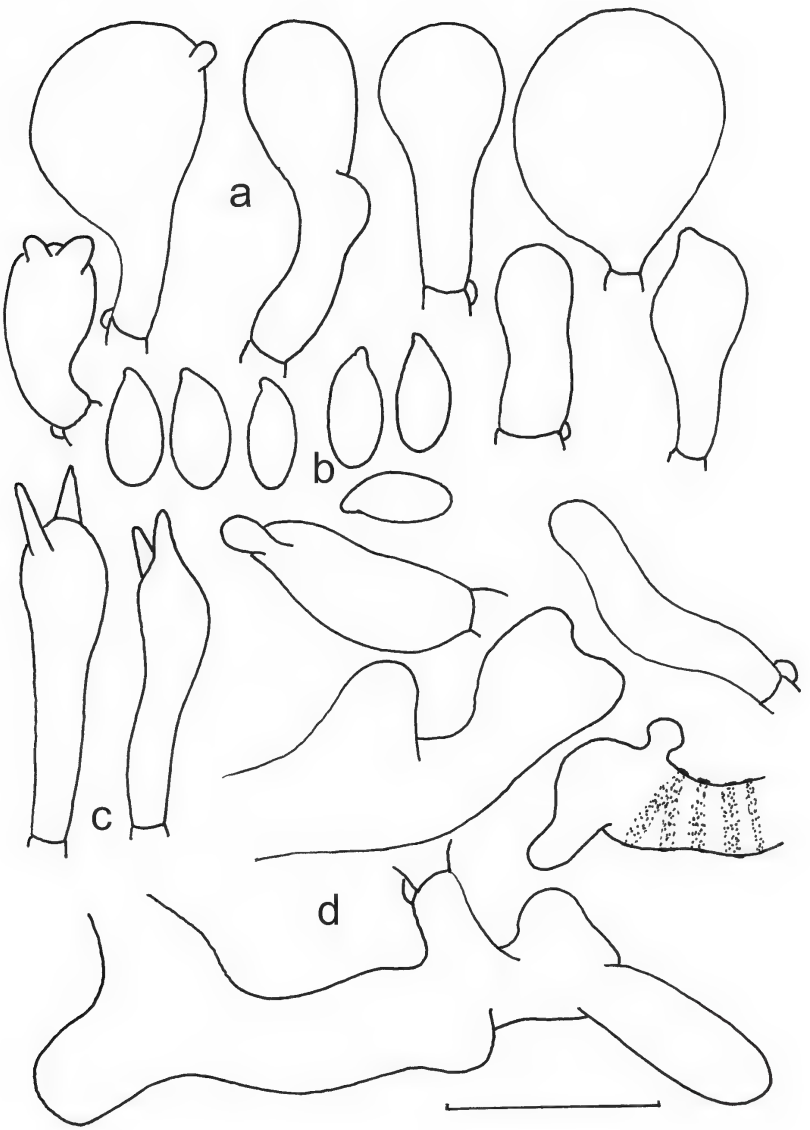

Fig. 3. *Gymnopus inusitatus* var. *cystidiatus* (holotype).
a. cheilocystidia, b. basidiospores, c. basidia, d. pileipellis. Scale bar = 20 μm.

at margin especially when old, brown to reddish brown (7–8E7) at centre, paler towards margin. Lamellae rather distant, L = 14–17, l = 2–3, emarginate and attached with tooth, ± ventricose, whitish, with concolorous edge. Stipe up to 35 × 0.75 mm (dry specimens), cylindrical, slightly broadened at apex, cylindrical to slightly clavate at base, smooth, ± concolorous with pileus at base with paler upper part; basal mycelium brown to reddish brown. Context without any distinct smell; taste not tested.

Basidiospores (10–)10.5–13 × 4.5–6.0 µm, average = 11.3 × 5.3 µm, E = 1.9–2.7, Q = 2.15 (40 spores, 2 basidiomes, 1 collection), ellipsoid-fusoid, pip-shaped, thin-walled. Basidia 26–33 × 7.5–8.5 µm, 2-spored, clavate. Basidioles up to 43 × 4.0–9.0 µm, cylindrical, clavate, subfusoid. Cheilocystidia 18–34(–40) × 7.0–12(–20) µm, clavate, subutriform, often capitate, sometimes irregular, smooth or with projection(s), thin-walled. Trama hyphae ± cylindrical, smooth or minutely incrusted, up to 12 µm wide. Pileipellis a cutis composed of cylindrical to slightly inflated elements, sometimes irregular or with projections, ± thin-walled, coarsely incrusted (zebroid), 3.0–10 µm wide hyphae with greenish brown incrustation in KOH; terminal cells cylindrical, clavate, fusoid, only minutely incrusted or smooth, often irregular or with projections; some elements transient to poorly developed dryophila-structure. Stipitipellis a cutis of parallel, cylindrical, slightly thick-walled, incrusted, up to 5.0 µm wide hyphae. Caulocystidia absent, scattered appressed to (sub)erect, cylindrical, rare terminal cells with scattered projections present. Clamp connections present.

Chemical reactions — No part of basidiocarps amyloid or dextrinoid.

Ecology & distribution— Between *Salix* and grass remnants in open sand calcareous dune near *S. cinerea* and *S. purpurea*. So far known only from the type locality.

Additional specimens examined — ***Gymnopus bisporus***: Spain, Catalonia, Girona, La Fonollera, Torroella de Mongrí, 1 Oct. 2000, J. Carbó (holotype of *Collybia bispora*, BCN–SCM B-4065, GenBank: JN247551, JN247555).

Gymnopus catalonicus: Spain, Catalonia, Barcelona, Can Ferrer, 23 Oct. 2000, J. Vila & X. Llimona (holotype of *Collybia catalonica*, BCN–SCM B-4057), GenBank: JN247552, JN247556.

Gymnopus inusitatus: Spain, Catalonia, Roses, Coll de la Perafita, 6 Oct. 1998, J. Vila & X. Llimona (holotype of *Collybia inusitata*, BCN–SCM B-4058), GenBank: JN247553, JN247557.

Discussion

Gymnopus inusitatus var. *cystidiatus* is characterized macroscopically (Fig. 2) by having basidiomes similar to *Mycetinis scorodonius*, and microscopically (Fig. 3) by rather large basidiospores, long, 2-spored basidia, well-developed clavate, subutriform, often capitate cheilocystidia, a pileipellis composed of

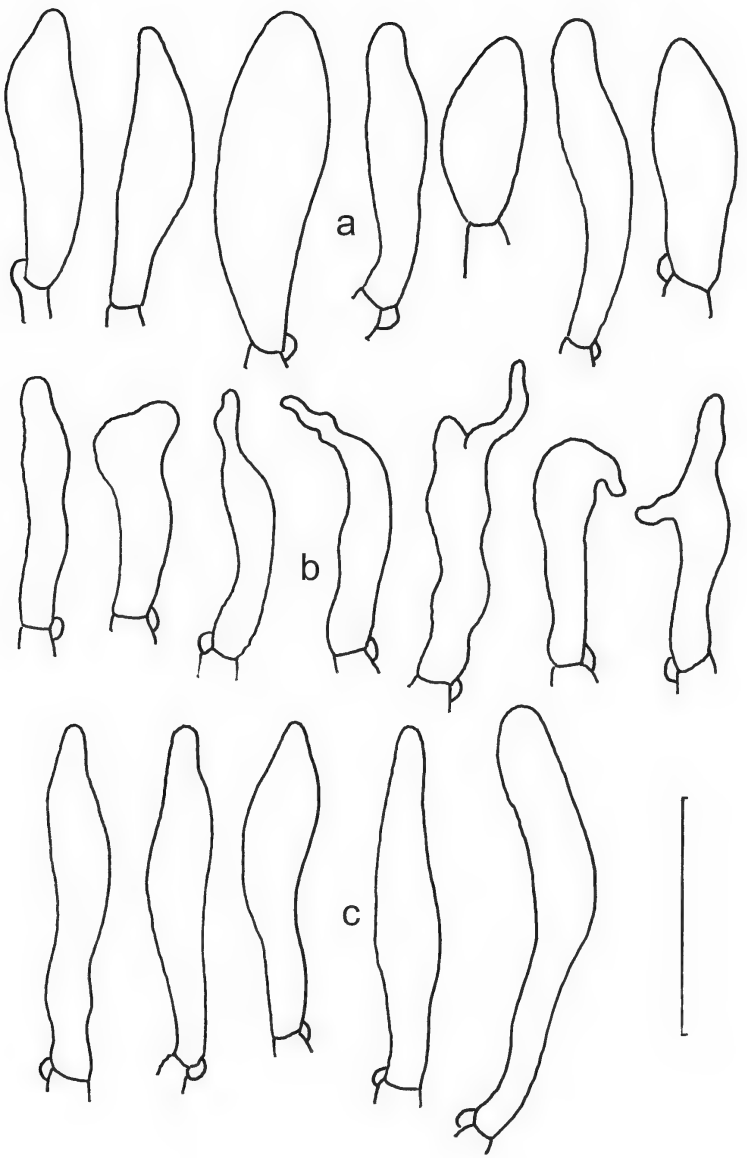

Fig. 4. *Gymnopus inusitatus* var. *inusitatus*. Marginal cells of lamellae (holotypes). a. *Collybia inusitata*, b. *C. bispora* and c. *C. catalonica*). Scale bar = 20 μm.

hyphae with greenish brown incrustation in KOH forming a cutis or only a poorly developed dryophila-structure, and cylindrical, clavate, fusoid, often irregular terminal cells, an absence of caulocystidia, and ecologically by growing in sandy grasslands under *Salix*.

In this group of bisporic taxa, Antonín & Noordeloos (2010) distinguished two species — *Gymnopus bisporus* (syn. *G. catalonicus*) with a brown to reddish brown (marginally paler) pileus and basidiospores measuring 9.0–11(–12) × 4.5–5.5(–6.0) µm and *G. inusitatus* with a reddish brown to violaceous brown pileus (cream to ochraceous-brown when drying), and slightly narrower basidiospores measuring 10–14 × 4.0–5.0 µm. Both species lack true cheilocystidia (Fig. 4). The basidiospore size variation in this group of taxa is distinctly wider than admitted by the current anatomic-morphological species concepts. The same situation was noted for European species in the *Psilocybe cyanescens* group (Borovička 2008, Borovička et al. 2010).

The most important diagnostic characters of *G. inusitatus* var. *cystidiatus* are the presence of well-developed cheilocystidia and a different. All three taxa synonymized under of *G. inusitatus* var. *inusitatus* were collected in xerothermic acidophilous stands with various *Cistus* species (Pérez-De-Gregorio & Carbó 2002, Vila & Llimona 2002). *Gymnopous inusitatus* var. *cystidiatus* was collected in calcareous sandy grasslands under *Salix*. Its type locality lies in the Great Hungarian Plain in the northwestern part of the Pannonic sand steppes where the open xeric grassland is dominated by the Pannonian endemic *Festuca vaginata* on dune tops and communities of *Salix rosmarinifolia* and other *Salix* species in the moist interdune depressions. The Hungarian collection represents the first record of *G. inusitatus* in central Europe.

Species from the *G. dryophilus* group differ from *G. inusitatus* by a well-developed dryophila-structure in the pileipellis, tetrasporic basidia, and smaller basidiospores. *Gymnopus erythropus* (Pers.) Antonín, Halling & Noordel. has red-brown coloured hairs on the stipe base like *G. inusitatus* but differs in basidiome colour, smaller basidiospores ((5.0–)5.5–8.0(–9.0) × 3.5–4.5(–5.0) µm), tetrasporic basidia, and differently shaped cheilocystidia (Antonín & Noordeloos 2010).

The macroscopically similar *Gymnopus earlei* Murrill from North America has a larger (≤ 35 mm broad) pileus, buff or pale orangish yellow (later orangish buff) lamellae, a more robust stipe (10–46(–90) × 2–5 mm), smaller basidiospores (5.6–7 × 2.8–3.5 µm), mostly tetrasporic basidia, and inconspicuous cheilocystidia (Halling 1983, Vilgalys & Miller 1983). *Gymnopus bicolor* A.W. Wilson et al. has similar cheilocystidia and a poorly developed dryophila-structure, but it has crowded lamellae, a larger (22–28 mm broad) pileus, a more robust stipe (30–42 × 2–3 mm), tetrasporic basidia, and smaller basidiospores (5.2–8 × 2.4–3.6 µm; Wilson et al. 2004).

Acknowledgments

The authors are very grateful to the curator of the BCN herbarium for a loan of the type specimens, to Zdeněk Pouzar (Prague, Czech Republic) for correcting the Latin diagnosis, and to Alexander Urban (University of Vienna, Vienna, Austria) and Alfredo Vizzini (Università degli Studi di Torino, Torino, Italy) for their critical review of the manuscript. Studies of the first author were financially supported by the Ministry of Culture of the Czech Republic (MK 00009486201).

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New records of *Pezizales* from Pakistan

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Abstract — *Geopora cooperi* f. *cooperi* and *Peziza succosa* have been described and illustrated morphologically and by amplifying ITS1-5.8S-ITS2 region of rDNA. Both taxa are new records from Pakistan.

Key words — ascocarp, ascospores, operculate ascus

Introduction

Pezizales are characterized by unitunicate asci that generally open by rupturing to form a terminal or eccentric lid or operculum (Hansen & Pfister 2006). Of the 1638 pezizalean species known, *Geopora* Harkn. and *Peziza* Dill. ex Fr. are represented by 13 and 104 species respectively (Kirk et al. 2008). From Pakistan three *Geopora* species — *G. arenicola* (Lév.) Kers, *G. arenosa* (Fuckel) S. Ahmad, *G. foliacea* (Schaeff.) S. Ahmad) — and eight *Peziza* species — *P. badiofusca* (Boud.) Dennis, *P. cerea* Bull., *P. gerardii* Cooke, *P. micropus* Pers., *P. pakistanica* (S. Ahmad) S. Ahmad, *P. repanda* Pers., *P. vesiculosa* Bull., *P. violacea* Pers.) — have been reported (Ahmad 1978; Ahmad et al. 1997).

This paper describes two taxa of *Pezizales* newly recorded from Pakistan. The specimens were first identified morphologically, after which the ITS1+5.8S+ ITS2 rDNA regions were amplified for comparison with GenBank sequences of related species. Eighty-seven taxa of *Pezizales* were previously reported from Pakistan; the addition of our recently collected *Geopora cooperi* f. *cooperi* and *Peziza succosa*) raises that total to 89.

Materials & methods

Collected ascocarps were photographed and necessary ecological data were recorded in field. The specimens were dried with the help of fan heater and preserved with tentative field No., for further studies. Apothecia were rehydrated and free hand sections were made in the laboratory. Microscopic (morpho-anatomical) characters were noted at 10× and 40× and drawings were made with the help of camera lucida.

DNA was extracted from dried ascomata, using Extract N. Amp.™ Plant kit (SIGMA). nrDNA of the ITS1-5.8S-ITS2 was amplified using fungal specific and universal primers pairs (ITS1F/ITS4 and ITS1/ITS4, respectively) under the following parameters: denaturation at 94°C for 4 min, then with 35 cycles of 45 s at 94°C, 45 s at 54°C and 1 min 30 s at 72°C, and a final amplification at 72°C for 2 min. Bidirectional sequencing of the purified PCR products was done by Macrogen Inc. (South Korea). ITS sequences were compared using BLAST in GenBank to confirm our identifications.

The specimens have been deposited in the *Pezizales* collection of LAH Herbarium, Department of Botany, University of the Punjab, Lahore, Pakistan. Sequences have been accessioned in GenBank.

Taxonomy

Geopora cooperi Harkn., Bull. Calif. Acad. Sci. 1: 168 (1885), f. ***cooperi*** PLATE 1

ASCOCARP: 5–5.5 cm broader, globose to oblong, irregularly convoluted, outer surface dark brown and covered with light brown hairs, inner surface pinkish white to white, highly convoluted, convolutions close but separate. HYMENIUM: 200–250 µm thick. ASCI: Long cylindrical, operculate, centric operculum, unitunicate, 8-spored, uniseriate, non-amyloid, tapering at base, (213–) 221–260 × (16–)19–21 µm. ASCOSPORES: Broadly oval-elliptical, hyaline, smooth, thin walled, with a large central guttule, cytoplasm dense around the guttule, (19–)20–23(–25) × 13–14 µm. Paraphyses: Long, slender, septate, swollen at the tips, nearly of same level as ascus, apical cell 14.5 µm broad. EXCIPULUM: Ectal excipulum: textura globulosa, 120 µm, 3–4 cells thick, cells oblong to circular in section, smooth, walls thick and dark brown, 24–40 × 18–27 µm, cells on the outside darker than the inner cells. Outer cells of the excipulum forming abundant, hyaline to light brown hairs, that are flexuous, branched, 11–13 µm wide, thick-walled, (walls up to 3 µm). Medullary excipulum of textura intricata, thin walled hyphae, hyaline to light brown.

MATERIAL EXAMINED: **PAKISTAN**: **GILGIT-BALTISTAN**, Fairy Meadows, 3036 m (9960ft) a.s.l., under *Pinus wallichiana* A.B. Jacks., solitary, on ground, 24 July 2010, A.N. Khalid TA-50. (LAH 240710; GenBank JN558642).

COMMENTS: *Geopora cooperi* differs from other epigeous *Geopora* species by producing truffle-like ascocarps with a small basal opening and a heavily folded layer (Tamm et al. 2010). It differs from most other truffle-like fungi because of the forcible discharge of ascospores: truffle spores are usually not forcibly expelled from the ascus. Mycorrhizal interaction of *Geopora cooperi* with *Pinus ponderosa* P. Lawson & C. Lawson and the orchid *Epipactis atrorubens* (Hoffm.) Besser has been established (Sheffereson et al. 2008). *Geopora cooperi* f. *cooperi* differs from *G. cooperi* f. *gilkeyae* Burds. in the narrower oval to elliptical ascospores (Burdsall 1968). Our DNA sequence analyses matched the 625 nucleotide ITS region (ITS1-5.8S-ITS2) from the Pakistani collection with that

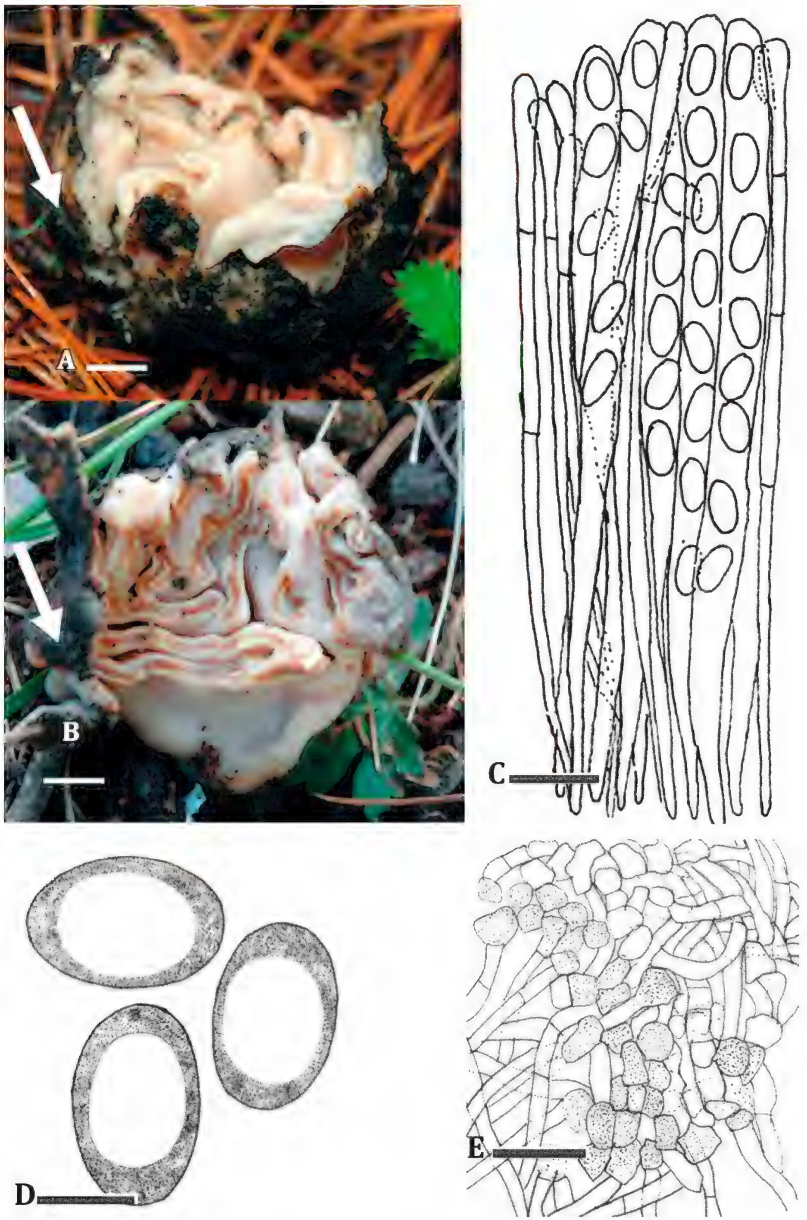

Plate 1. *Geopora cooper* f. *cooperi:* A–B. Different views of apothecia (arrow indicates outer hairy layer of fruiting body). C. Section of hymenium showing asci and paraphyses. D. Ascospores. E. Ectal excipulum. Scale bars: A = 1.5 cm; B = 1 cm; C = 40 µm; D = 13.5 µm; E = 24 µm

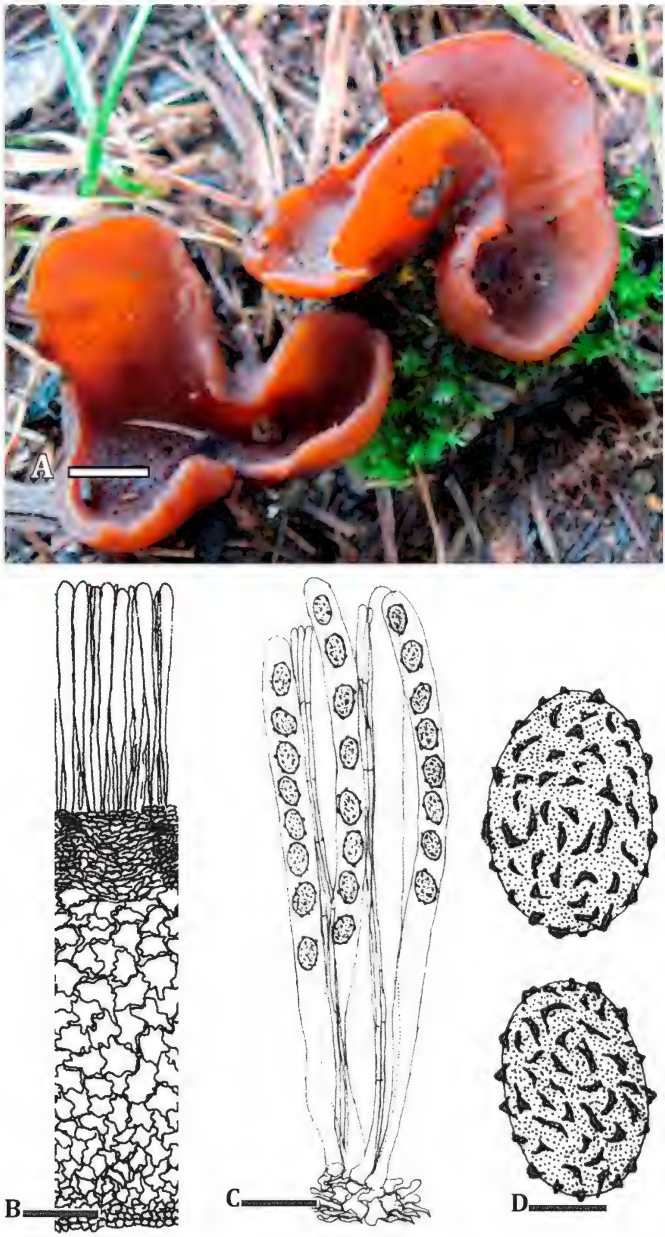

Plate 2. *Peziza succosa:* A. Apothecia. B. Section of apothecium showing hymenium, subhymenium and excipulum. C. Asus and paraphysis. D. Ascospores. Scale bars: A = 1.1 cm; B = 190 µm; C = 49.1 µm; D = 9.5 µm.

from *Geopora cooperi* f. *cooperi* (FR694202.1) at 98% similarity and 90% Query coverage, which we consider sufficient to refer our collection to this taxon.

Peziza succosa Berk., Ann. Mag. Nat. Hist. 6: 358 (1841) PLATE 2

APOTHECIA: Usually gregarious, cupulate, deeply concave, medium-sized, disc concave at first, hemispherical and later slightly irregular, 2.5–3cm in diam., sessile to subsessile, margins entire turning inwards, purplish brown hymenium with slight yellowish tinge, outer surface slightly lighter than hymenium, fresh apothecia exudes yellowish juice when damaged, Subhymenium plectenchymatous. HYMENIUM: up to 304–315 µm. ASCI: Unitunicate, operculate, 8-spored, amyloid at tip, cylindrical slightly narrow near base with flexuous stalk, 260–300 × 17–19µm. ASCOSPORES: Uniseriate, elliptical, ornamented with warts, biguttulate,19–20 × 10–11(–12) µm. PARAPHYSES: Slender, straight, septate, 3.5–4 µm in diam. EXCIPULUM: Ectal excipulum 95 µm thick, textura globulosa to angularis, cells hyaline to slightly brown, smaller thin-walled cells also present; medullary excipulum 285 µm thick epidermoid to polygonal cell, hyaline, thin-walled, 14–25 µm in diam.

MATERIAL EXAMINED: **PAKISTAN**: **KHYBER PAKHTUNKHWA**, Himalayan Moist Temperate Forests, Khanspur, 2575 m (8205 ft) a.s.l., gregarious, on ground, mossy substrate, 21 Aug 2010, T. Ashraf TA-134 (LAH 210810; GenBank JN588568).

COMMENTS: *Peziza succosa* has strongly amyloid asci, with the bluing reaction strongest at the apex, although no pronounced bluing ring is present. With other ectomycorrhizal fungi, *P. succosa* has been reported in deciduous forests and is widespread in Europe, North America (Hansen et al. 2001, Michell 2006), and Asia (DH Pfister, pers. comm.). ITS sequence analyses of our collection showed it to be 99% identical with *P. succosa* (DQ200840.1) from Fredericksburg Have, Zealand, Denmark.

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A new *Hysterostomella* species from the Cerrado in Brasília National Park

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Abstract — A new *Hysterostomella* species found on leaves of *Connarus suberosus* is illustrated and described as *Hysterostomella connari.*

Key words — *Ascomycota*, biodiversity, *Parmulariaceae*, taxonomy, tropical fungi

Introduction

Parmulariaceae, including 34 genera of foliicolous biotrophic fungi, occurs primarily in the Neotropics and Paleotropics (Inácio & Cannon 2008, Kirk et al. 2008). The type genus of the family, *Parmularia* Lév. and its type species *P. styracis* Lév., was described on *Styrax ferrugineus* (Léveillé1846), a plant species endemic to the Brazilian Cerrado. For decades this family was set aside with occasional contributions (Müller & von Arx 1973, von Arx & Müller 1975, Barr 1987, Sivanesan 1970, Sivanesan & Hsieh 1989, Sivanesan et al. 1998). Recently the type species of all accepted genera were re-examined, consolidating our understanding of the family on the basis of morphological features (Inácio 2003, Inácio & Cannon 2003, 2008). Now a new species belonging to the genus *Hysterostomella*, previously observed by Sales (2003) and Silva et al. (2006) from the Brazilian Cerrado, is formally described and illustrated.

The revision by Inácio (2003) of *Hysterostomella* Speg., type species *H. guaranitica* Speg., included a re-description of several species. The genus is characterized by subcuticular internal stromata when host cells are incorporated by the infective mycelium. Sometimes these structures are subepidermal, connected to superficial stromatic ascomata by peg-like or tubular hyphal filaments. The discoid multilocular stromatic ascomata contain spherical or elongate locules dispersed in various orientations (Inácio 2003, Inácio & Cannon 2008). *Hysterostomella* is close to *Cycloschizon* Henn., which differs by

forming internal stromata that become erumpent via a column with a wart-like expanded top containing locules distributed in complete or incomplete rings. *Dothidasteroma* Höhn., also close to *Hysterostomella*, differs mainly by forming flat internal stromata, never subepidermal, that connect to the superficial ascomata by either bundles of dark colored hyphae, or single hyphae.

Materials & methods

Leaf samples of *Connarus suberosus* containing dark brown to black crustose fungal structures were collected from different areas of Cerrado in Parque National, Brasília. These were pressed and dried for 3–4 days at ± 50°C, and deposited in the Mycological Collection of the Herbarium UB. The leaves were observed under a stereomicroscope to describe the lesions and location and characteristics of the fungal colonies. For the morphological studies, each specimen was mounted on slides using lacto-glycerol/cotton blue or glycerol KOH/basic phloxine, to observe the internal structures. Pieces of the dried material containing stromata were re-hydrated for 4–12 h using an aqueous solution containing 10% ethanol and 0.1% Tween-20. Then 7–15 µm thick sections were produced using a freezing microtome (MICROM GMBH, HM 500 OM, Micron Laborgeräte, Walldorf, Germany). A compound light microscope (Zeiss Ultraphot III, Carl Zeiss, Oberkochen, Germany) was used for the photographic documentation and to obtain morphometrical data. Pieces of leaves with one or more lesions showing representative samples of fruiting bodies were examined with SEM after treatment in a 0.1 M sodium cacodylate buffer (pH 7.4) containing 2% glutaraldehyde for at least 24 h. The samples were dehydrated in an increasing acetone concentration series (15%, 30%, 50%, 75%, 100%; 15 min per concentration). Leaf pieces were then dried at the critical point before being covered by a thin layer of gold in a sputter coater for 2 min. Finally, the samples were observed in a scanning electron microscope (Jeol, model JSM 840-A E).

Taxonomy

Hysterostomella connari Inácio, Pereira-Carvalho, E.S.C. Souza, H.B. Sales & Dianese **sp. nov** FIGS 1–21

MycoBank MB 561556

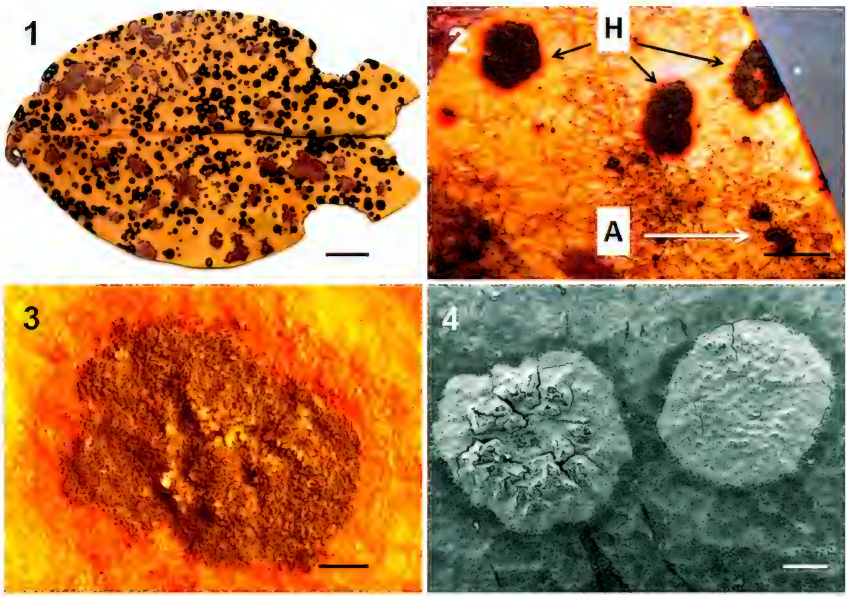
Species haec ab Hysterostomella tetracerae *differt conidiis non fasciatis et grandioribus.*

TYPE: BRAZIL. Distrito Federal, Brasília, Parque Nacional, near Parada do Cristal, on leaves of *Connarus suberosus* Planch. (*Connaraceae*), 18 Jan. 2010, leg. *Carlos Antonio Inácio* (**holotype**, UB Mycol. Coll. 21492).

ETYMOLOGY: referring to the host genus.

LESIONS 3–12 mm diam, black, circular encircled by a reddish linear band, adaxially more evident. Colonies sparse, containing black circular or irregular superficial stromata that are discoid, mostly epiphyllous, crustose, sheltering conidiomata, spermogonia and/or ascomata.

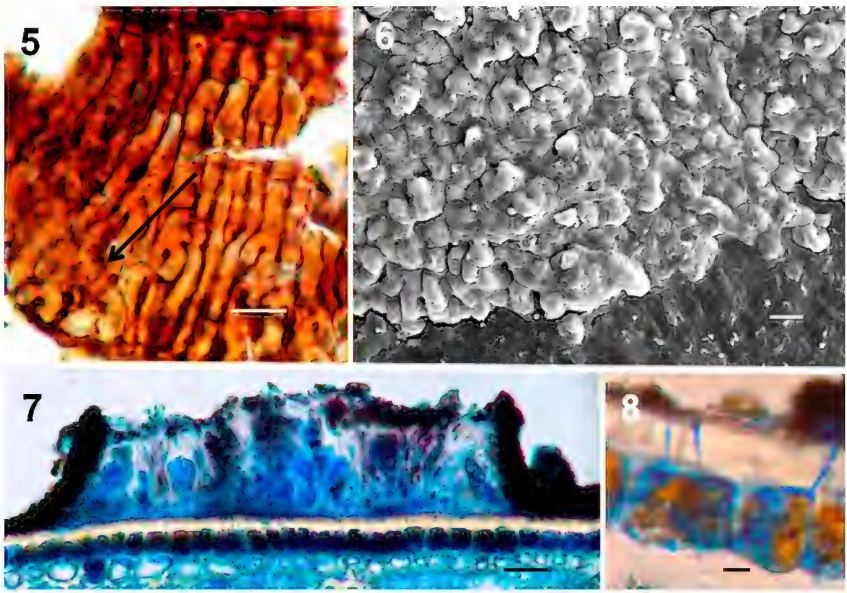
INTERNAL PSEUDOSTROMATA as mycelium with colourless to reddish-brown angular cells invading and incorporating one or more host cell layers, to 30 µm



Figs 1–4. *Hysterostomella connari* on leaves of *Connarus suberosus*. 1. Adaxial colony shown as a group of stromatic ascomata circular to irregular in shape. 2. Crustose stromatic ascomata (H) together with a colony of an *Asterina* species (A). 3. A stromatic ascoma bordered by a reddish band (bar). 4. An older opened ascoma (left) and another still closed (right) seen in SEM. Scale bars: 1 = 10 mm; 2 = 2 mm; 3, 4 = 0.5 mm.

deep. Superficial stromata 0.1–3.5 mm diam., 50–113 × 150–680 µm in vertical section, subepidermal, erumpent, brown to dark brown, shiny, with laciniate edges; upper surface composed of textura prismatico-radiata, the hyphae with brown prismatic cells that proliferate dichotomously towards the margins to form a circular or irregular 1–2 cell-layered multilocular shield that houses the sexual and asexual fruiting bodies; rather prominent, opening by irregular slits; upper wall 10–30 µm thick, brown to dark brown, with surface consisting of 5–7 µm diam brown cells; lower wall 30 µm thick, brown to dark brown, covering the host epidermal cells, dense, bearing a basal cushion of mingled colourless, light brown to brown hyphae, bearing the asci or lining the internal surface of the conidiomata and spermogonia. Mycelium internal. Hyphae colourless to pale brown, 1–2 µm diam, septate, branched, penetrating and occupying the epidermal and first layers of mesophyll, sometimes with coiled intracellular structures; to 100 µm deep.

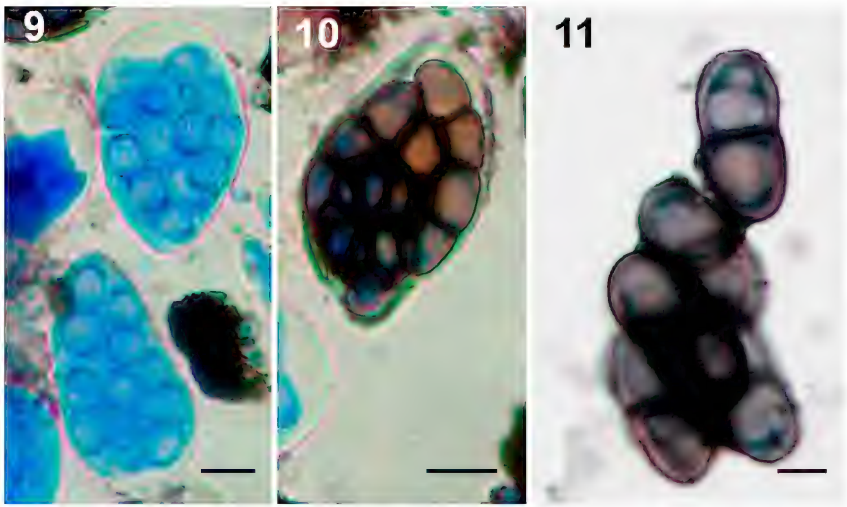
Conidiomata included in the superficial stromata, gregarious, intermixed with the ascomata and spermogonia, globose to subglobose, opening by diametral or irregular fissures, uni- or multilocular, 40–160 µm high in vertical



Figs 5–8. *Hysterostomella connari* on leaves of *Connarus suberosus*. 5. Superficial textura of a stromatic ascoma, with arrows showing the dichotomous proliferation of component hyphae. 6. Surface of an ascoma seen under SEM. 7. Vertical section through an ascoma showing parallel asci and branched sterile filaments, and transcuticular hyphae connecting the flat portion of the pseudostroma with the superficial ascoma. 8. Detail of the interconnection between the components of the internal pseudostroma with the superficial ascoma. Scale bars: 5, 6, 8 = 10 µm; 7 = 50 µm.

section, with a brown to dark brown upper wall; UPPER WALL dense, 10–25 (–30) µm thick, composed of textura prismatico-angularis; CELLS 3–7 µm diam.; CONIDIOMATAL LOCULES 25–150 × 120–610 µm, single or sometimes multiple separated by a stromatic wall, with conidiogenous cells and sterile filaments attached to the inner face of the upper locular wall; CONIDIOGENOUS CELLS 8–20(–25) × 3–7 µm, ampulliform, cylindrical to lageniform, colourless to pale brown, smooth, holoblastic; CONIDIA 9–16 × 6–10 µm, initially colourless, becoming pale brown to brown, guttulate, obovoid to widely clavate, aseptate, smooth, with a truncate base. SPERMOGONIA structurally and dimensionally similar to the conidiomata; SPERMOGENOUS CELLS 2–7 × 2–3 µm, phialidic, hyaline, cylindrical; SPERMATIA 3–5 × 1–2 µm, aseptate, hyaline, bacilliform.

ASCOMATA 50–95 × 155–580 µm, intra-stromatic, occurring separately from, or mixed with conidiomata and/or spermogonia occupying the same stromatic crust, containing parallel asci and sterile filaments on a basal cushion of colourless to light brown hyphae covered by a thin gelatinous layer; YOUNG ASCI variable in shape, cylindrical, clavate to broadly clavate, with a short



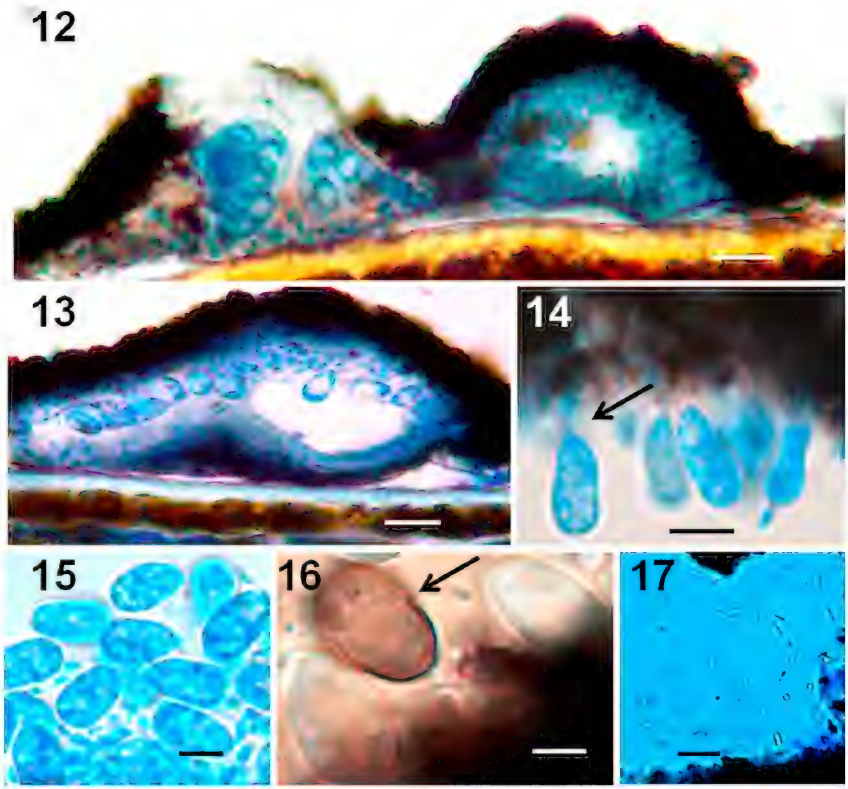
Figs 9–11. *Hysterostomella connari* on leaves of *Connarus suberosus*. 9. Immature asci. 10. A mature bitunicate ascus with dark brown ascospores. 11. Ascospores. Scale bars: 9–11 = 10 µm.

pedicel, thick-walled particularly towards the apex where, before spores are visible, a sub-apical chamber is present; FULL-SIZED ASCI showing 60–70 × 18–30 µm, 6- to 8-spored with ascospores arranged in one to three rows, broadly-clavate, thick-walled particularly in the upper part, collapsing after spore release showing a large apical crack in a typically fissitunicate fashion; ASCOSPORES 17–21 × 7–11 µm, becoming light to dark brown, 1-septate, guttulate, covered by a mucilaginous sheath, ellipsoidal, round at both ends, narrower at the septum, tapering towards the lower tip; INTERASCAL FILAMENTS septate, smooth, colourless to light brown, thin-walled, 2–4 µm diam, filiform, round or slightly attenuate at the tip, branched above the middle portion, immersed in a gelatinous matrix.

ADDITIONAL SPECIMENS EXAMINED: **BRAZIL. DISTRITO FEDERAL, BRASÍLIA, Parque Nacional**: 2 km North from Parada do Cristal—on leaves of *Connarus suberosus*, 24 Jan. 2007, leg. Rafael Guimarães, collection number 6 (UB Mycol. Coll. 20.420); Parada do Cristal´s pathway—on leaves of *Connarus suberosus*, 06 Feb. 2006, Siomara Vasconcelos da Silva (UB Mycol. Coll. 20.115); Unknown sites within Parque Nacional—08 Jan. 1996, Zuleide Martins Chaves 302 (UB Myc. Coll. 10.656) & 321 (UB Myc. Coll. 10.675); 15 Jan.1996, Zuleide Martins Chaves 350 (UB Myc. Coll. 10.738); 24 Jan. 1996, Mariza Sanchez 1476 (UB Myc. Coll. 10.911); 20 Jan. 2010, Gustavo Puga Lemes (UB Myc. Coll. 21529) and Carlos Antonio Inácio (UB Mycol. Coll. 21516)

Discussion

Inácio (2003) and Inácio & Cannon (2008) have partially revised *Hysterostomella*. All species are assumed to show clear host specificity, and no



Figs 12–17. *Hysterostomella connari* on leaves of *Connarus suberosus*. 12. An ascoma (left) beside a spermogonium (right). 13. A conidioma with a typically inverted hymenium. 14. Holoblastically formation of a conidium (arrow). 15. A group of immature hyaline conidia. 16. Mature dark brown aseptate conidium (arrow). 17. Spermatia. Scale bars: 12, 13 = 50 µm; 14, 15, 17 = 10 µm; 16 = 5 µm.

Hysterostomella species has previously been recorded associated with members of the *Connaraceae*. Among the species with re-examined type materials, only *H. tetracerae* (Höhnel 1909) and *H. gymnosporiae* (Hansford 1947) have a conidial anamorph similar to that found in *H. connari*. However both differ because their conidia have a clear hyaline median band. In the remaining species there are no records of anamorphs. Spermogonia as shown in *H. connari* were previously reported only in *H. tetracerae,* which, however, differs additionally by its larger ascomata (88–188 µm diam.), darker and easily discernible lower ascomatal wall, and narrower asci. *Hysterostomella gymnosporiae* forms more prominent ascomata (170–250 µm high) and more restricted internal pseudostromata than *H. connari*.

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Penicillium mallochii and *P. guanacastense*, two new species isolated from Costa Rican caterpillars

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Abstract — Twenty-five strains of monoverticillate *Penicillium* species were isolated from dissected guts and fecal pellets of leaf-eating caterpillars reared in the Área de Conservación Guanacaste, Costa Rica, or from washed leaves of their food plants. Phylogenetic analyses of β-tubulin, nuclear ribosomal internal transcribed spacer (ITS), cytochrome c oxidase subunit 1, translation elongation factor 1-α and calmodulin gene sequences revealed two phylogenetically distinct, undescribed species closely related to *P. sclerotiorum*. *Penicillium mallochii* was isolated from *Rothschildia lebeau* and *Citheronia lobesis* (*Saturniidae*) and their food plant *Spondias mombin* (*Anacardiaceae*) and *P. guanacastense* from *Eutelia* sp. (*Noctuidae*). Both fungi produce greenish conidial masses and orange pigments in agar culture, have smooth-walled, monoverticillate conidiophores with moderately vesiculate apices, and globose to subglobose conidia. The species morphologically resemble *P. sclerotiorum* but differ subtly in vesicle width and conidial shape.

Key words — *Penicillium* subg. *Aspergilloides*, sect. *Sclerotiora*, DNA barcoding, All Taxa Biological Inventory (ATBI)

Introduction

In 2001, a microbial observatory was established in Sector Santa Rosa of the UNESCO World Heritage Site, Área de Conservación Guanacaste, northwestern Costa Rica, to culture microbiota (including fungi) associated with caterpillars reared on the tree *Spondias mombin* as a model for elucidating ecological roles of insects and associated microbes in a tropical forest ecosystem.

The fungi isolated from dissected caterpillar guts and fecal pellets were the subject of an MSc thesis (Urb 2006) and included many *Penicillium* strains that required detailed taxonomic study to identify. Several strains were identified as the common and widespread biverticillate *Penicillium citrinum* Thom, but a significant number had unbranched, monoverticillate conidiophores and colonies reminiscent of *P. sclerotiorum* J.F.H. Beyma (Pitt 1979).

As part of the Canadian Barcode of Life Network, we explored DNA barcoding of *Penicillium* species using cytochrome oxidase 1 (COX1; Seifert et al. 2007) and the proposed fungal DNA barcode, the internal transcribed spacer (ITS) of the nuclear ribosomal DNA. In this paper, two new species, *P. mallochii* and *P. guanacastense* are formally described from the Área de Conservación Guanacaste, as part of a broader revision of the *P. sclerotiorum* complex (Rivera & Seifert 2011). The fungi were characterized using cultural and microscopic characters, DNA barcode data, and additional DNA sequence data from β-tubulin (BENA), translation elongation factor 1-α (TEF1-α), and calmodulin (CMD) genes.

Materials & methods

Isolates

Caterpillars of *Rothschildia lebeau* and *Citheronia lobesis* (*Saturniidae*) feeding on *Spondias mombin* (*Anacardiaceae*), *Cochlospermum vitifolium* (*Bixaceae*), or *Exostema mexicanum* (*Rubiaceae*) were collected by Joel Díaz, Felipe Chavarría-Díaz, and Maria Garcia in the tropical dry forest of Sector Santa Rosa of Área de Conservación Guanacaste, northwestern Costa Rica. Caterpillars were surface sterilized with dH_2O and ethanol and guts were removed aseptically and, when possible, partitioned into foregut plus midgut and hindgut sections. Gut contents or feces (obtained from caterpillars kept in sterile Petri dishes) were suspended in sterile H_2O and serially diluted in sterile dH_2O, then plated on soil extract-rose bengal agar (Bills & Foster 2004). Fungal colonies were transferred to Malt Extract Agar (MEA, Samson et al. 2004) until pure cultures were obtained. Cultures are preserved in the Canadian Collection of Fungal Cultures (CCFC, Table 1) and ex-types are also deposited in the CBS-KNAW Fungal Biodiversity Centre culture collection (CBS), Utrecht, the Netherlands. Cultures lacking DAOM numbers are preserved in the personal collection of K. Seifert (KAS) or R.G. Thorn (RGTHC). Details for cultures of other species are given by Rivera & Seifert (2011).

BLAST searches of ITS and BENA sequences suggested that the new species were related to *Penicillium sclerotiorum*. When possible, we obtained five strains per species of this clade (Peterson 2000) for comparison, including *P. sclerotiorum*, *P. adametzii* K.M. Zalessky, *P. bilaiae* Chalab., *P. herquei* Bainier & Sartory and *P. hirayamae* Udagawa. Strains were obtained from CBS and the USDA-ARS, National Center for Agricultural Utilization Research culture collection (NRRL).

Morphology

For standardized descriptions, spore suspensions in 0.1% semi-solid agar were inoculated at three points on Czapek Yeast Agar (CYA, Pitt 1979) with added micro-

nutrients (Samson et al. 2004), and MEA in 9 cm polystyrene Petri dishes, and incubated at 25 °C for 7 d in the dark (Samson & Pitt 1985). To determine whether sclerotia could be induced, some strains were incubated for up to 6 months in incident light at room temperature on Oatmeal Agar (OA, Samson et al. 2004). Colony colors were determined using Kornerup & Wanscher (1978); alphanumeric color codes refer to this work. Colony photographs were taken with a Nikon CoolPix E 5000 camera with incandescent lighting and a copy stand.

Microscopic examinations employed an Olympus BX 50 light microscope on material removed from MEA colonies and mounted in 85% lactic acid. Microphotographs were taken with an Evolution MP Camera and microscopic characters were measured from digital images using Image-Pro Plus v. 6 (Media Cybernetics, MD, U.S.A.). For each strain, ten phialides, stipes, metulae and vesicles were measured manually, and 25 automated measurements were made for conidia.

DNA isolation, PCR amplification, DNA sequencing and phylogenetic analyses

Detailed protocols are provided in the revision of the *P. sclerotiorum* complex by Rivera & Seifert (2011), along with details of PCR and sequencing of some strains, and only a summary of relevant details is provided here. DNA was extracted from agar cultures using UltraClean™ Microbial DNA Isolation Kits (MO BIO Laboratories, Inc., Montreal, Canada) following the manufacturer's protocol. Genes were amplified by PCR and sequenced with the following primers: BENA Bt2a and Bt2b (Glass & Donaldson 1995); ITS ITS1 and ITS4 (White et al. 1990); COX1 PF and AR (Seifert et al. 2007); TEF1-α EF1c and EF6 (Peterson et al. 2004); CMD CMD5 and CMD6 (Hong et al. 2006).

PCR reactions were performed in 10 µl reaction mixtures with 1 µl genomic DNA, 1X PCR Buffer, 0.1 mm of dNTPs, 0.08 µM of each primer, and 0.5X Taq polymerase, in a TGradient (Biometra, Montreal, Canada) or a Genius (Techne, Duxford, Cambridge) thermocycler. Sequencing reactions were performed directly from PCR products in both directions, with templates precipitated by ethanol/EDTA/sodium acetate precipitation. Sequences were determined on an ABI 3130XL Genetic Analyzer (Applied Biosystems, Foster City, California, USA).

Consensus sequences were assembled using Sequencher v. 4.8 (Genes Codes Corporation, MI, USA). Alignments were constructed using the online version of MAFFT v. 6 (Katoh et al. 2009), and adjusted to optimize homology using BioEdit 7.0.9 (Hall 1999). Maximum parsimony (MP) analyses were performed using heuristic searches in PAUP v. 4 (Swofford 2002) with the tree bisection-reconnection (TBR) branchswapping algorithm. Maximum likelihood (ML) analyses were performed using Phylogenies for Maximum Likelihood (PhyML) v. 2.4.4 (Guindon et al. 2010). Tree searches for each alignment were run under the nucleotide substitution model obtained from ModelTest 3.7, using the Akaike Information Criteria (AIC) (Posada and Crandall, 1998), and starting from a tree obtained for branch swapping using the modified neighbor joining algorithm BIONJ (Gascuel 1997) as implemented by PhyML. Bayesian inference (BI) analyses were performed using MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001), under the same models selected for ML. All Bayesian analyses were performed using random starting trees, and for each gene, four chains were run for one million generations, sampling every 100 generations, generating 10,001 trees, with the first 2,500 discarded as 'burnin' for each chain.

Table 1. *Penicillium mallochii* and *P. guanacastense* strains used in this study.
All originate in Área de Conservación Guanacaste, Santa Rosa, Costa Rica.

Penicillium species	Accession number	ACG number	Caterpillar	Food Plant	GenBank Accession numbers				
					ITS	benA	tef-1α	cmd	cox1
mallochii	daom 239917*	03-rgthc-903	none	*S. mombin*	JN626104	JN625973	JN626152	JN626016	JN626065
	daom 239918	01-rgthc-144	*Rothschildia lebeau* (entire gut)	*S. mombin*	JN626105	JN625974	JN626153	JN626017	JN626066
	daom 239919	02-rgthc-474	*Citheronia lobesis* (mg)	*S. mombin*	JN626106	JN625975	JN626154	JN626018	JN626067
	daom 239920	03-rgthc-895	*R. lebeau* (hg)	*S. mombin*	JN626107	JN625976	JN626155	JN626019	JN626068
	daom 239921	03-rgthc-900	*R. lebeau* (fg, mg)	*S. mombin*	JN626108	JN625977	JN626156	JN626020	JN626069
	daom 239922	02-rgthc-386	*R. lebeau* (frass)	*S. mombin* ¹	JN626109	JN625978	JN626157	JN626021	JN626070
	daom 239924	02-rgthc-607	*C. lobesis* (fg, mg)	*C. vitifolium*	JN626110	JN625979	JN626158	JN626022	JN626071
	daom 239925	02-rgthc-599	*C. lobesis* (fg, mg)	*C. vitifolium*	JN626112	JN625980	JN626159	JN626023	JN626072
	daom 239926	02-rgthc-383	*R. lebeau* (frass)	*S. mombin*	JN626111	JN625981	JN626160	JN626024	JN626073
	daom 239927	01-rgthc-311	*R. lebeau* (entire gut)	*S. mombin*	JN626113	JN625982	JN626161	JN626025	JN626074
	daom 239928	02-rgthc-676	*C. lobesis* (fg, mg)	*C. vitifolium*	JN626114	JN625983	JN626162	JN626026	JN626075
	daom 239929	03-rgthc-893	*R. lebeau* (hg)	*S. mombin*	JN626115	JN625984	JN626163	JN626027	JN626076
	kas 2597	01-rgthc-294	*R. lebeau* (entire gut)	*S. mombin*	JN626116	JN625985	JN626164	JN626028	JN626077
	kas 2601	02-rgthc-481	*C. lobesis* (mg)	*S. mombin*	JN626117	JN625986	JN626165	JN626029	JN626078
	kas 2606	02-rgthc-679	*C. lobesis* (fg, mg)	*C. vitifolium*	JN626118	JN625987	JN626166	JN626030	JN626079

Penicillium species	Accession number	ACG number	Caterpillar	Food Plant	GenBank Accession numbers				
					ITS	benA	tef-1α	cmd	cox1
mallochii (continued)	kas 2612	03-rgthc-911	none	*S. mombin*	JN626119	JN625988	JN626167	JN626031	JN626080
	kas 2615	02-rgthc-387	*R. lebeau* (frass)	*S. mombin*	JN626120	JN625989	JN626168	JN626032	JN626081
	kas 2630	02-rgthc-389	*R. lebeau* (frass)	*S. mombin*	JN626121	JN625990	JN626169	JN626033	JN626082
	kas 3183	03-rgthc-891	*R. lebeau* (hg)	*S. mombin*	JN626122	JN625991	JN626170	JN626034	JN626083
	kas 3186	03-rgthc-770	*C. lobesis* (entire gut)	*S. mombin*	JN626123	JN625992	JN626171	JN626035	JN626084
	kas 3187	03-rgthc-894	*R. lebeau* (hg)	*S. mombin*	JN626124	JN625993	JN626172	JN626036	JN626085
	kas 3188	02-rgthc-621	*R. lebeau* (fg, mg)	*E. mexicanum*	JN626125	JN625994	JN626173	JN626037	JN626086
	kas 3190	02-rgthc-627	*R. lebeau* (fg, mg)	*E. mexicanum*	JN626126	JN625995	JN626174	JN626038	JN626087
	kas 3191	03-rgthc-896	*R. lebeau* (fg, mg)	*S. mombin*	JN626127	JN625996	JN626175	JN626039	JN626088
	kas 3193	03-rgthc-890	*R. lebeau* (hg)	*S. mombin*	JN626128	JN625997	JN626176	JN626040	JN626089
guanacastense	daom 239912*	02-rgthc-462	*Eutelia* sp. (entire gut)	*S. mombin*	JN626098	JN625967	JN626146	JN626010	JN626059
	daom 239913	02-rgthc-317	*Eutelia* sp. (entire gut)	*S. mombin*	JN626099	JN625968	JN626147	JN626011	JN626060

* Abbreviations: daom = Canadian Collection of Fungal Cultures; kas = personal culture collection of Keith Seifert; rgthc = accession numbers associated with the Guanacaste ATBI; fg = foregut; mg = midgut; hg = hindgut; * = ex-type strain. [1] *S = Spondias; C. = Cochlospermum; E. = Exostema*

All sequences for the two new species are deposited in GenBank with the accession numbers JN625955–JN626181 (Table 1). Details of strains of other species, and the GenBank accession numbers for their sequences, are provided by Rivera & Seifert (2011).

Results

Representative ML trees for each of the five genes (ITS, *cox1*, benA, cmd and tef1-α) are presented in Fig. 1. The MP and BI trees are not shown but differences in topology are discussed, and support values for each analysis are mapped onto the ML trees and included in Table 2. A combined gene tree is included in the revision of the entire *P. sclerotiorum* complex (Rivera & Seifert 2011).

Table 2. Statistical support for the monophyletic clades representing *Penicillium mallochii* and *P. guanacastense* in five gene data alignments. *

		P. mallochii	*P. guanacastense*
ITS	MP bootstrap (%)	99	100
	ML probability	0.998	1.00
	BI posterior probability	1.00	1.00
cox1	MP bootstrap (%)	85	100
	ML probability	0.986	0.996
	BI posterior probability	0.53	1.00
tef1-α	MP bootstrap (%)	91	100
	ML probability	0.975	1.00
	BI posterior probability	0.87	1.00
benA	MP bootstrap (%)	100	100
	ML probability	0.998	1.00
	BI posterior probability	1.00	1.00
cmd	MP bootstrap (%)	100	100
	ML probability	1.00	1.00
	BI posterior probability	1.00	1.00

*analyzed by Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian Inference.

Both *P. mallochii* and *P. guanacastense* received strong support as distinct monophyletic clades in all analyses of all five genes (Table 2). The MP benA trees revealed two groups of strains within *P. mallochii* 86% and 70% bootstrap support, which are absent in the other gene trees. These groups had 0.866 and 0.814 ML probabilities, and BI posterior probabilities of 0.74 and 0.66. Long branch lengths subtending these two clades on the MP tree appear to be an artifact, sometimes experienced with maximum parsimony analyses.

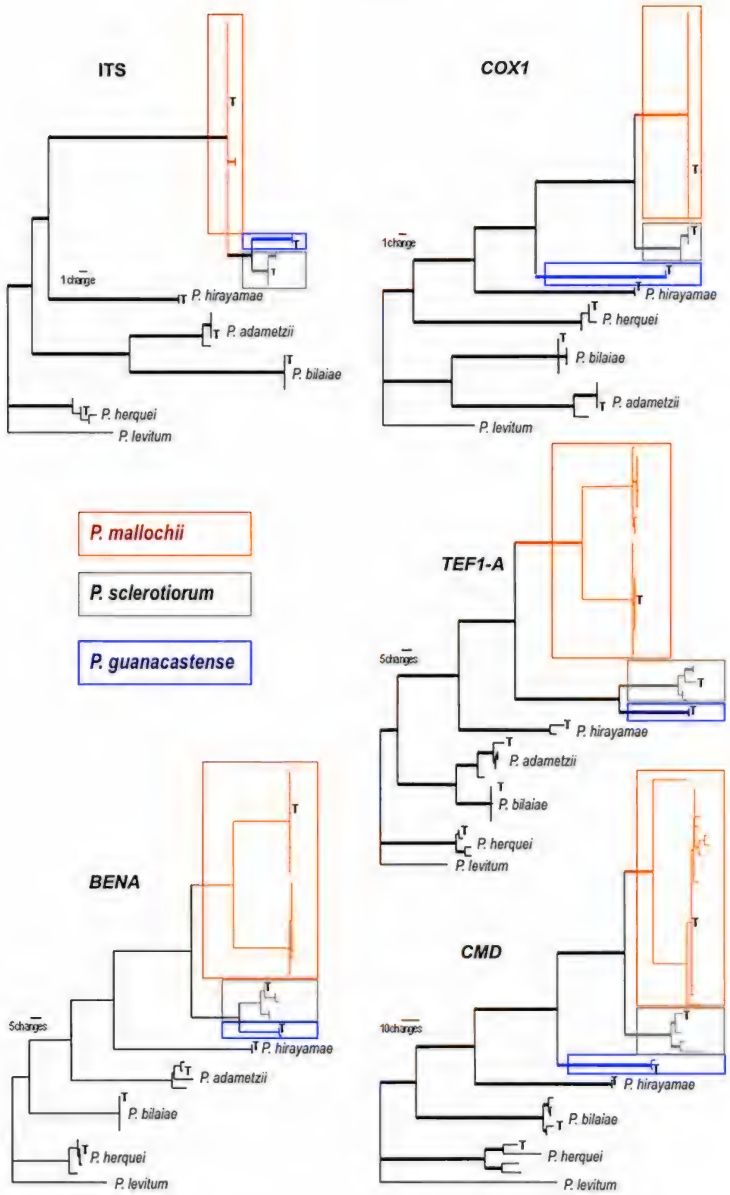


Fig 1. Maximum likelihood (ML) gene trees for ITS, cox1, tef1-α, benA and cmd data sets. The negative-log likelihood (–lnL) of the ML trees are –1497.83287, –1610.84725, –3299.83157, –2712.50962, –3454.53854. Branches with ML-bootstrap support >95%, MP-bootstrap support >95%, and Bayesian Inference partition probabilities >0.99 are thickened.

Taxonomy

Penicillium mallochii K.G. Rivera, Urb & Seifert, **sp. nov.** FIGS 2, 4–8

MycoBank MB 563043

Growth on CYA 29–39 mm, on MEA 24–35 mm diam. after 7 days. Conidiophores monoverticillate, stipes 50–380 × 2–4 µm, terminal vesicle 4–6 µm wide, rarely with a single metula 15–45 × 1.5–2 µm. Phialides 7–10(16) × 2–3(4) µm. Conidia (sub)globose, 2.5–3.5 × 2–2.5 µm.

TYPE: Costa Rica, Santa Rosa, Área de Conservación Guanacaste, isolated from leaf of *Spondias mombin*, 2003, leg. Joel Díaz, Felipe Chavarría-Díaz, and Maria Garcia no. 03-RGTHC-903, isol. M. Urb (**Holotype**, DAOM 239917, dried culture on Blakeslee's MEA; ex-type culture, CCFC 239917).

ETYMOLOGY: '*mallochii*' named for Prof. David Malloch, retired mycologist from the University of Toronto, now a visiting fellow at the Natural History Museum in St. John, New Brunswick, an expert on ascomycetes, and a mentor to KGR, RGT and KAS.

Colonies on CYA after 7 days at 25°C: typically (24–)29–39 mm diam.; dense and velutinous, sometimes with aerial mycelium in the center; moderately sulcate, with 4–7 grooves, often wrinkled in the center; approximately 1 mm deep; conidia Turquoise Grey to Greenish Grey (24–25D2–3), concentric rings of different shades of these colors present; clear yellow exudate droplets produced moderately in some strains, absent in others; vivid orange soluble pigment present in some strains, absent in others; 2–3 mm of white mycelia at the margin, margin entire; reverse deep Yellow (4A8), Reddish Orange (4A6–7) or Golden Yellow to Orange (5–6A–B6–8) in the center, Light to Pastel Yellow (3–4A4–5) at the margins.

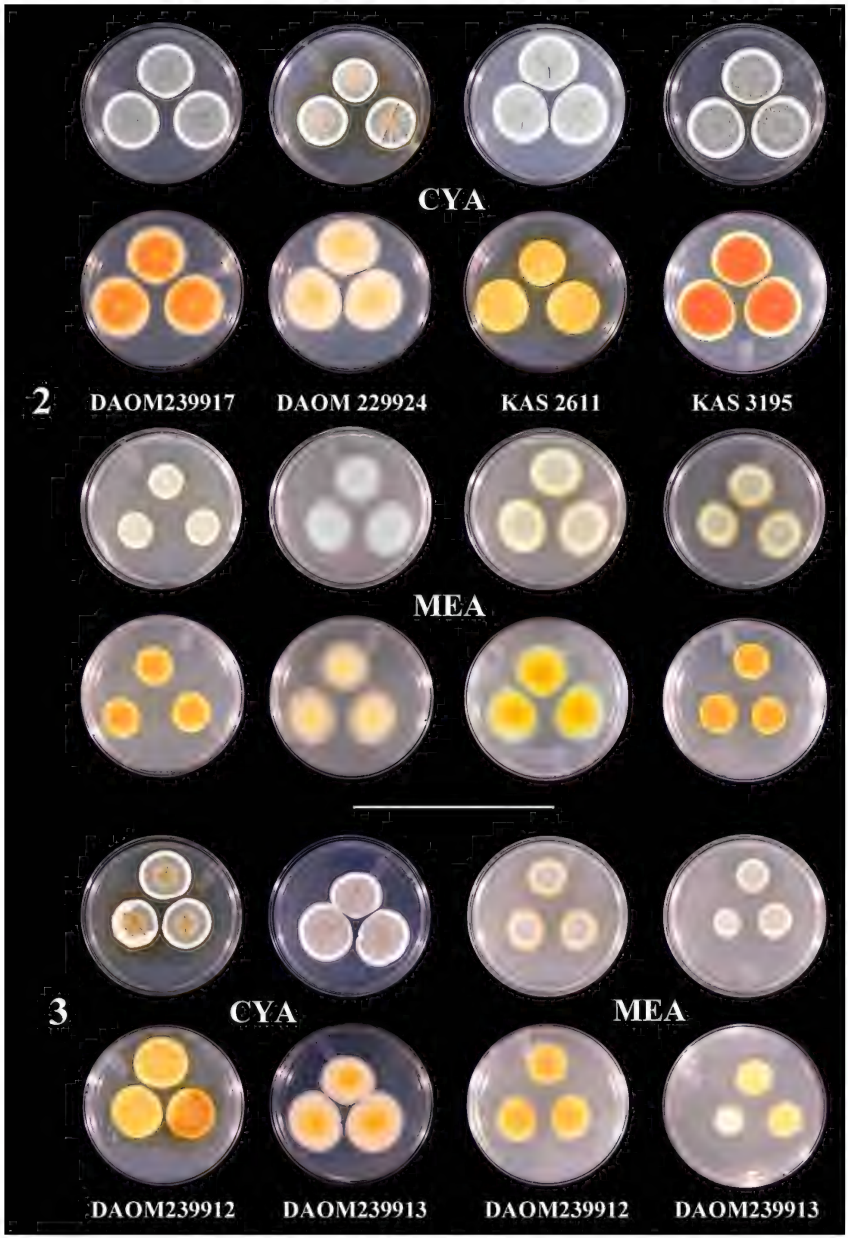
Colonies on MEA after 7 days at 25°C: (19–)24–35 mm diam; planar, velutinous, with no aerial mycelium; conidia Greyish Green to Dull Green (25–30B–E3–7), sometimes forming crusts that dislodge when the colony is disturbed, no exudates or soluble pigments present; reverse Greyish Green (30C5), Greyish Yellow (1–2B–C3–8), Yellow (2–3A6–7), Deep Yellow (4A8) or Orange (6A–B6–8), near the margins paler shades of these colors; margin entire.

Conidiophores monoverticillate, borne from agar surface, stipes septate, 53–380 × 2–4 µm, smooth to finely roughened; mostly unbranched, but in some strains ~10% of the conidiophores have single branches 15–45 × 1.5–2 µm; vesicles 4–6 µm diam (mean for different strains = 4.5–6.0 ± 0.71 µm, n = 25). Phialides 7–10(–16) × 2–3(– 4) µm, ampulliform with a collula. Conidia globose to subglobose, finely roughened, 2.5–3.5 × 2–2.5 µm (range of means for different strains = 2.6–3.4 ± 0.01 × 2.3–2.4 ± 0.01, n = 25), mean L/W ratio 1.15:1.

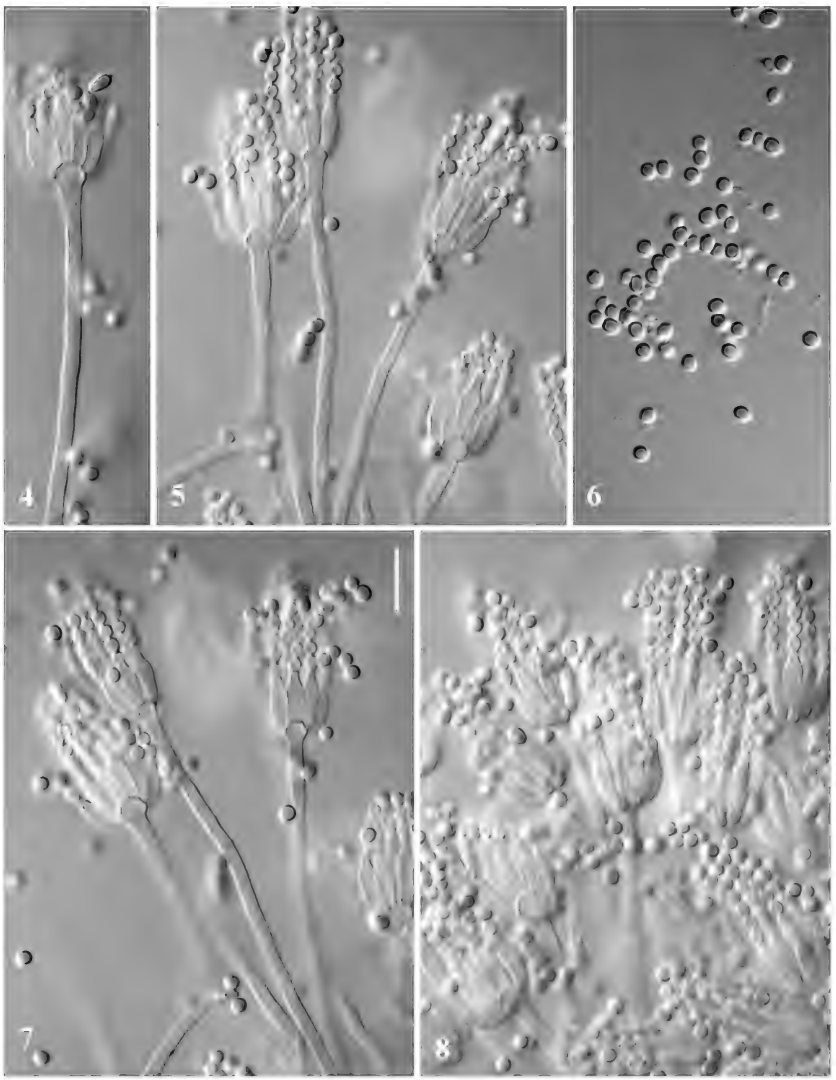
DISTRIBUTION: Área de Conservación Guanacaste, Sector Santa Rosa, Guanacaste Province, northwestern Costa Rica.

HABITAT: *Rothschildia lebeau* and *Citheronia lobesis* (*Saturniidae*) caterpillar guts and faeces, and leaves of *Spondias mombin* (*Anacardiaceae*).

ADDITIONAL MATERIAL EXAMINED: See TABLE 1.



Figs 2–3. Seven day old cultures of *Penicillium mallochii* (2) and *P. guanacastense* (3) on CYA and MEA, showing variation in colony characters.



Figs 4–8. *Penicillium mallochii,* photomicrographs from the holotype. 4–5 and 7–8. Monoverticillate conidiophores. 6. Conidia. Scale bar = 10 µm.

Penicillium guanacastense K.G. Rivera, Urb & Seifert, **sp. nov.** Figs 2, 9–12

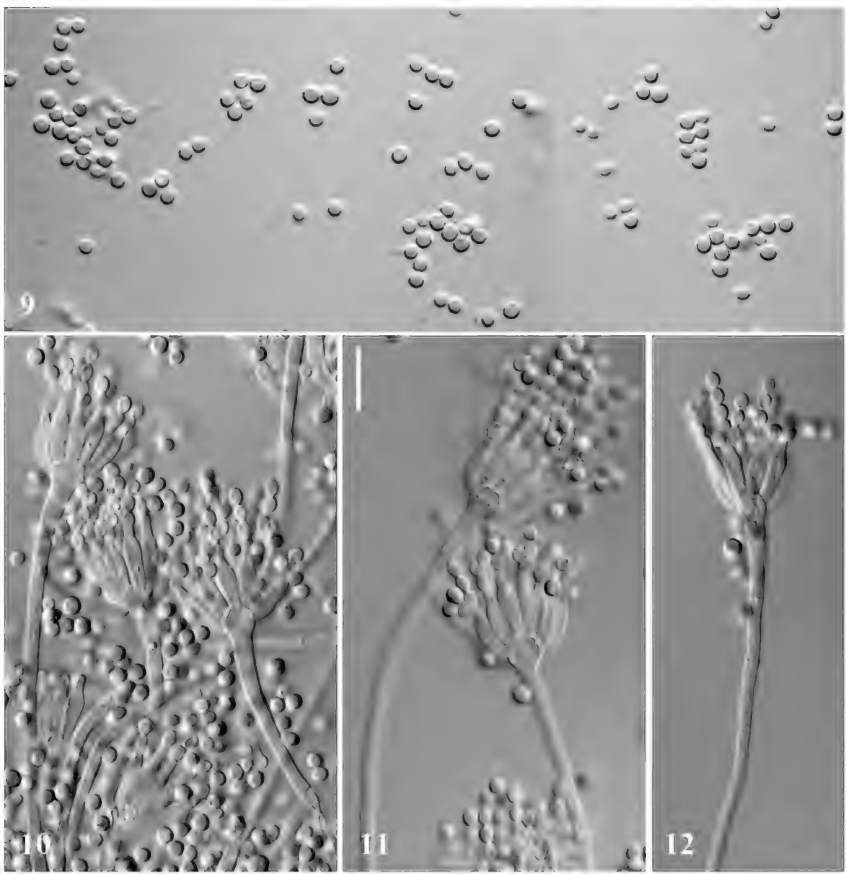
MycoBank MB 563044

Growth on CYA 25–33 mm, on MEA 29–33 mm diam. after 7 days. Conidiophore monoverticillate or rarely with one metula, stipes 85–100 × 2.5–3 µm, terminal vesicle 4–5.5 µm wide. Phialides 8.5–12 × 2–3.5 µm. Conidia globose, 2.5–3.0 µm diam.

TYPE: Costa Rica, Santa Rosa, Área de Conservación Guanacaste, isolated from *Eutelia* sp. reared on leaf of *Spondias mombin*, 2002, leg. Joel Díaz, Felipe Chavarría-Díaz, and Maria Garcia no. 02-RGTHC-462, isol. M. Urb (**Holotype**, DAOM 239912, dried culture on Blakeslee's MEA; ex-type culture, CCFC 239912).

ETYMOLOGY: '*guanacastense*' named for the Área de Conservación Guanacaste, where the fungus was discovered.

Colonies on CYA after 7 days at 25°C: 25–33 diam, dense, velutinous, sulcate, with 5–8 grooves, sometimes with sectors with poor sporulation; conidia Greenish Grey (25–30B–E2), with yellow concentric ring near the margins and a bluish grey concentric ring towards the center, clear exudate droplets produced moderately and yellow soluble pigments produced by one strain; 2–3



FIGS 9–12. *Penicillium guanacastense,* photomicrographs from the holotype. 9. Conidia. 10–12. Monoverticillate conidiophores. Scale bar = 10 μm.

mm of white mycelium at the margin, margin entire; reverse Orange Yellow to Orange (4–6B7–8).

Colonies on MEA after 7 days at 25°C: 29–33 mm diam., planar, velutinous, no aerial mycelia observed; conidia Greenish Grey (25–30B–E2), sometimes forming crusts that dislodge when the colony is disturbed, with no exudates or soluble pigments present; reverse Yellowish White (2–4B2), Orange (6B7–8) or Light Brown (6–7D4–8), with paler shades of these colors near the margin.

Conidiophores monoverticillate on MEA, borne directly from agar surface; stipes finely roughed, septate 85–100 × 2.5–3 µm; unbranched or sometimes up to ~10% have single branches 15–21 × 2.5–3 µm; vesicles 4–5.5 µm (mean 4.5–4.65 ± 0.5 µm). Phialides 8.5–12 × 2–3.5 µm, ampulliform with distinguishable collula. Conidia globose, finely roughened, 2.5–3 µm diam (range of means for different strains 2.7–2.8 ± 0.01 × 2.5–2.6 ± 0.01 µm, n=25), mean L/W ratio 1.1:1.

Distribution: Área de Conservación Guanacaste, Sector Santa Rosa, Guanacaste Province, northwestern Costa Rica.

Habitat: Guts of *Eutelia* caterpillar (*Noctuidae*) feeding on foliage of *Spondias mombin* (*Anacardiaceae*).

Additional material examined: See Table 1.

Discussion

Penicillium mallochii and *P. guanacastense* are phylogenetically related and morphologically very similar to *P. sclerotiorum*. All three species have vesiculate, monoverticillate conidiophores and vivid orange to red colony reverses on CYA. Morphological characters place both species in *Penicillium* subg. *Aspergilloides* ser. *Glabra* (Pitt 1979), and the recently erected, phylogenetically and morphologically defined sect. *Sclerotiora* (Houbraken & Samson 2011). All species in this series have long, monoverticillate conidiophores with a swollen or vesiculate apex. The conidiophores of *P. sclerotiorum* sensu stricto are conspicuously vesiculate (mean 3.6 ± 0.4 µm), while those of *P. mallochii* and *P. guanacastense* are only moderately vesiculate, but with the vesicles of *P. mallochii* (3.1 ± 0.2 µm) being slightly more swollen than those of *P. guanacastense* (3.0 ± 0.3 µm). Furthermore, *P. sclerotiorum* has ellipsoidal conidia, while *P. mallochii* and *P. guanacastense* have globose to subglobose conidia. Some strains of *P. sclerotiorum* produce pale colored sclerotia (Pitt 1979), which were not observed in any of our *P. mallochii* and *P. guanacastense* strains.

There are ecological differences among these three species. *Penicillium mallochii* strains were isolated from saturniid caterpillars and leaves of their food plant *Spondias mombin*, whereas *P. guanacastense* was isolated from noctuid caterpillars, also feeding on *Spondias mombin*. In contrast, *P. sclerotiorum* strains have mostly been isolated from the soil and the air.

Based on our five-gene analysis of multiple strains of each species, *P. mallochii, P. guanacastense,* and *P. sclerotiorum* are phylogenetically distinct species. Each species was resolved as a strongly supported monophyletic group with each gene, with each kind of analysis, except for the ITS MP and BI analyses, which did not resolve *P. mallochii* from the branch that grouped *P. guanacastense* and *P. sclerotiorum*.

In the study of microfungi isolated from guts and frass of two species of caterpillars feeding on the tree *Spondias mombin*, *Penicillium* was the fourth most commonly isolated of the 48 identified genera, comprising 88 of 835 isolates (Urb 2006). Twenty-four strains of *P. citrinum* were isolated, and this was the most frequent species isolated from guts of *Citheronia lobesis* (with three β-tubulin variants, represented by GenBank accession numbers JN637993–JN637995). A similar number of strains of *P. mallochii* were isolated from *Rothschildia lebeau* (Urb 2006). Whether these species are phylloplane inhabitants or endophytes of *S. mombin* leaves is unknown, and the significance of their frequent association with these caterpillars remains a subject for future research.

Acknowledgments

We are grateful for the critical reviews of the manuscript by R.A. Samson and S.W. Peterson, to Rafik Assabgui for processing sequences, and the curators of the CBS, DAOM, and NRRL culture collections for providing strains for this study. Walter Gams kindly corrected our Latin diagnoses for an early draft. The fungal collection and isolations were funded by a subcontract to RGT under the U.S. National Science Foundation grant NSF DEB-0084224 to Anna-Louise Reysenbach (Portland State University) and NSF DEB 0515699 to Daniel Janzen. The taxonomic studies were funded by Genome Canada through the Ontario Genomics Institute, NSERC, and other sponsors listed at www.BOLNET.ca.

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Two new species of *Terriera* from Yunnan Province, China

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Abstract —Two new species of *Terriera* are described and illustrated from Yunnan Province, China. *Terriera rotundata* on leaves of *Quercus* sp. is recognized by its more or less circular ascomata in vertical section. *Terriera petrakii* on *Smilax bracteata* is similar to *Lophodermium camelliicola* but differs from the latter by the pale conidiomata and ellipsoidal conidia and the well-developed textura prismatica between the covering and the basal stroma.

Key words —*Ascomycota*, *Rhytismatales*, *Fagaceae*, *Smilacaceae*

Introduction

The genus *Terriera* B. Erikss. was first segregated from *Lophodermium* Chevall. based mainly on paraphyses swollen at the tip to form an epithecium and the lack of lip cells (Eriksson 1970). Johnston (1988, under *L. multimatricum*; 1989, under *L. minus*) considered ascomatal development and structure as important features for *Terriera*. Molecular analyses support *Terriera* as monophyletic (Ortiz-García et al. 2003, Lantz et al. 2011). Many *Lophodermium* species with ascomatal structures typical of *Terriera* have been transferred to *Terriera* (Johnston 2001, Ortiz-García et al. 2003). Of the 18 species recognized worldwide, only two species have been reported for China (Fröhlich & Hyde 2000, Yang et al. 2011). We describe here two new species of *Terriera* from Yunnan Province, China.

Materials & methods

Hand sections of different thickness of ascomata were prepared using a razor blade and mounted in water, Melzer's reagent, 5% KOH, or 0.1% (w/v) cotton blue in lactic acid. For the observation of ascomatal outlines in vertical section, sections were pretreated in water before being mounted in lactic acid or cotton blue. Gelatinous sheaths surrounding ascospores and paraphyses were observed in water or cotton blue. Ascospore contents were drawn based on observations in water mounts. Measurements

were taken of 20 ascospores and asci for each specimen from tissues mounted in 5% KOH or Melzer's reagent.

Taxonomy

Terriera rotundata C.L. Hou, **sp. nov.** FIGS. 1–5

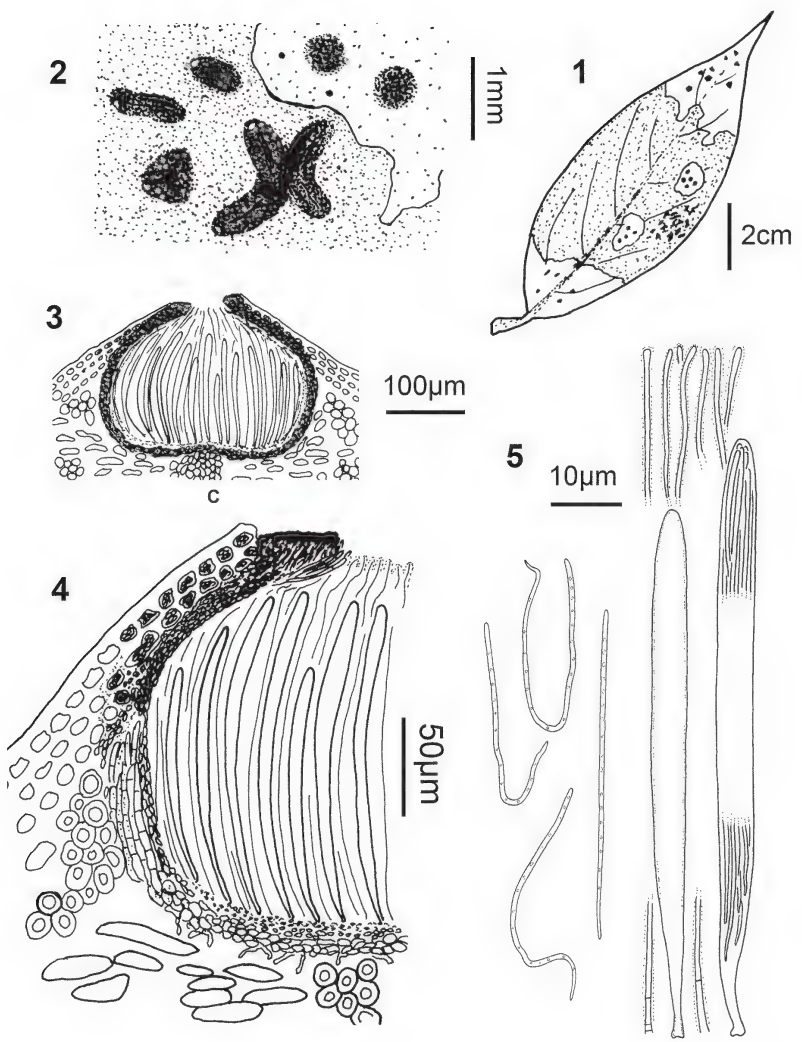
MycoBank 561752

T. rotundata differs from the known species in *Terriera* by its more or less globular ascomata in vertical section.

TYPE: China. Yunnan, Chuxiong, Zixishan, alt. ca. 2400 m, on fallen leaves of *Quercus* sp. (*Fagaceae*). 2 August 2001, C.-L. Hou et al. 172 (**Holotype** BJTC 201102).

ETYMOLOGY: *rotundata* refers to the almost rounded shape of the ascoma as seen in vertical section.

ASCOMATA developing on fallen leaves, epiphyllous, occasionally hypophyllous, not associated with bleached, pale brown areas. Ascomata (450–)600–900 × 330–450 µm, elliptical but often strongly curved at maturity, occasionally triangular, young ascomata more or less elliptical in outline, black, shiny, with a blurred margin, both ends rounded and often with pale spots, the central part of the ascomata strongly raising above the surface of the substrate, opening by a longitudinal split or occasionally by teeth. Immature, unopened ascomata macroscopically visible as two black areas separated from each other by a broad, pale longitudinal zone. In median vertical section, ascomata 140–180 µm deep in the host tissue, more or less circular in section. COVERING STROMA consistently 28–35 µm thick, composed of an outer layer of host cuticle, epidermal and hypodermal cells filled with dark brown, thick-walled fungal cells 2–3 µm diam., and an inner layer formed by 3–4 rows of hyaline, thin-walled, angular cells. Horizontal portion of the covering stroma 20–30 µm long, 10–25 µm thick, composed of periphysoids arising from the inner part of the covering stroma, their tips forming a hard black crust. A triangular space in section between the covering stroma and the basal stroma is filled with vertically arranged rows of prismatic, hyaline to slightly brown, thin-walled cells 6–9 × 3–5 µm. BASAL STROMA medium to well-developed, ± flat, composed of brown to dark brown textura globulosa-angularis, 15–20 µm thick. EXCIPULUM and LIP CELLS absent. SUBHYMENIUM 6–10 µm thick, composed of small cells 2–3 µm diam. PARAPHYSES 120–140 × 1 µm, filiform, septate or aseptate, unbranched, slightly swollen at the apex, embedded in gelatinous sheaths. ASCI ripening sequentially, 90–120 × 4–5.5 µm, narrow-cylindrical, thin-walled, J^-, without circumapical thickening, discharging spores through a small apical hole, 8-spored. ASCOSPORES fasciculate, 70–90(–95) × 0.8–1 µm, filiform, hyaline, aseptate, with numerous guttules, gelatinous sheaths not seen. Liberated ascospore often strongly bent.



Figs 1–5. *Terriera rotundata* on leaves of *Quercus* sp. 1. A leaf bearing ascomata. 2. Ascomata observed under the dissecting microscope (top right corner, two immature ascomata of *Coccomyces* sp. 3. Ascoma in vertical section. 4. Structure of an ascoma in vertical section. 5. Paraphyses, an ascus after the liberation of the ascospores, a mature ascus with ascospores, and discharged ascospores.

Zone lines only present between colonies of this and taxonomically distinct species.

Conidiomata not observed.

Comments — *Terriera rotundata* is distinctive within the genus because of its more or less globular ascomata in vertical section. The developmental pattern in *T. rotundata* is similar to that of *T. minor* (Tehon) P.R. Johnst. in vertical section with the textura prismatica between covering and basal stroma composed of vertically oriented cells (Johnston 1988, 1989, 2001). However, the shelf-like ridge along ascomatal opening typical of *Terriera* is poorly developed in *T. rotundata*. In addition, liberated ascospores of *T. rotundata* are often strongly bent and lack gelatinous sheaths.

Terriera petrakii C.L. Hou, **sp. nov.** Figs. 7–13

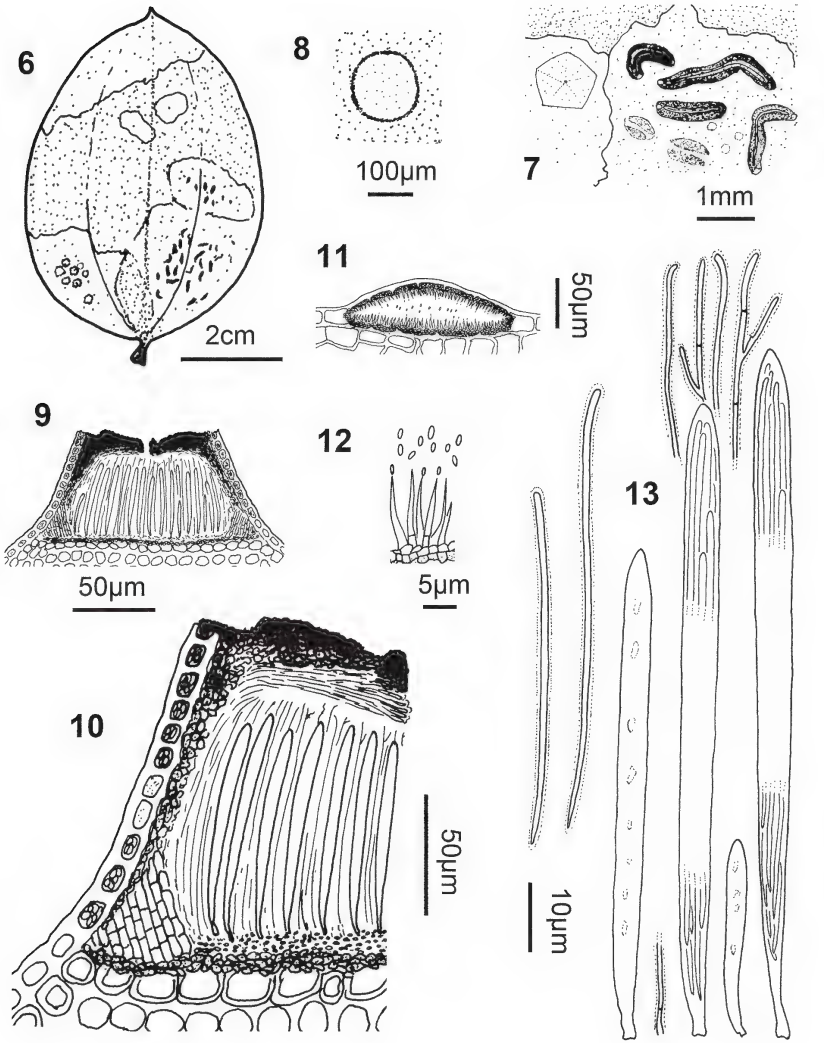
MycoBank 561753

T. petrakii is similar to *T. minor* but differs by conidiomata and conidia.

Type: China, Yunnan, Chuxiong, Zixishan, alt. ca. 2400 m, on fallen leaves of *Smilax bracteata* Presl (*Smilacaceae*), 2 August 2001, C.-L. Hou et al. 170 (**Holotype**, BJTC 201103).

Etymology: In honour of Franz Petrak, a well-known mycologist who studied a similar but undescribed species, "*Lophodermium smilacinum*" Petr.

Ascomata developing on leaves, epiphyllous, occasionally hypophyllous, in slightly bleached pale brown areas, associated with thin, black zone lines. Ascomata (600–)800–1300 × 380–450 μm, elongate-elliptical, often strongly curved or triangular, often coalesced, black in the central part, margin blurred, shiny, both ends often with pale spots, the central part of the ascomata strongly raising above the surface of the substrate, forming an elongate, flat zone at the opening, lips absent, opening by a longitudinal split. Immature, unopened ascomata macroscopically appearing as two black areas separated by a broad, pale longitudinal zone, young ascomata more or less elliptical in outline. In median vertical section, ascomata subepidermal, 120–150 μm deep, ± trapezoid in section. Covering stroma 23–30 μm thick over its entire extent, consisting of an outer layer of host epidermal cells filled with dark brown, angular fungal cells, a layer of dark brown textura angularis of different thickness and an inner layer of hyaline, angular cells, horizontal portion of the covering stroma 80–100 μm long and 15–25 μm thick, composed of black textura angularis with cells 4–6 μm diam. The triangular zone in section between the covering stroma and the basal stroma composed of thin-walled, cylindrical cells arranged in vertical rows. Basal stroma, medium to well-developed, ± flat, composed of brown to dark brown textura globulosa-angularis, 10–15 μm thick. Excipulum present, formed by paraphysis-like cells 1–2 μm diam., thickened near the



Figs 1–7. *Terriera petrakii* on leaves of *Smilax bracteata*. 6. A leaf bearing ascomata, conidiomata and zone lines. 7. Ascomata observed under the dissecting microscope (right, an ascoma of *Coccomyces occultus*). 8. A conidioma observed under the dissecting microscope 9. Ascoma in vertical section. 10. Structure of an ascoma in vertical section. 11. Structure of a conidioma in vertical section. 12. Conidiogenous cells and conidia. 13. Paraphyses, a young ascus, two mature asci with ascospores, and discharged ascospores.

opening, embedded in a gelatinous matrix. Subhymenium consisting of textura angularis with cells 2–4 µm diam., 6–10 µm thick. Paraphyses 100–125 × 1 µm, filiform, septate, branched, or unbranched, not swollen at the apex. Asci ripening sequentially, 85–110 × 4–5 µm, cylindrical, thin-walled, J–, without circumapical thickening, discharging spores through a small apical hole, 8-spored. Ascospores fasciculate, (60–)70–85 × 0.8 µm, filiform, hyaline, aseptate, with a thin gelatinous sheath.

Conidiomata in pale brown areas near the ascomata, round, pale, with conspicuous black perimeter line, 140–300 µm diam., opening by ostioles. In vertical section, conidiomata intraepidermal, 40–55 µm deep. Upper layer composed of host cuticle, remains of host epidermal cells, and hyaline textura angularis with cells 3–5 µm diam. Basal layer 50–58 µm thick, composed of light brown textura angularis. Conidiogenous cells ovoid-cylindrical, 9–13 × 1.5–2 µm tapering towards the apex, hyaline. Conidia broadly ellipsoidal, hyaline, 1.5–2 × 1 µm.

Additional specimen examined —**United States, Hawaii:** Olinda Pipe Line, on fallen twigs of *Smilax* sp., coll. C.L. Shear & N.E. Stevens, det. F. Petrak, 29 December 1928 (W 618).

Comments — The ascomatal structure of *Terriera petrakii* is very similar to that of *T. minor,* which differs in shorter, uncurved ascomata, broader 0–1-septate ascospores, a less well developed excipulum, and an unknown anamorph. The conidioma shape and structure and ascospore shape in *T. petrakii* are very similar to those of *Lophodermium camelliicola* Minter (which should be transferred to *Terriera*). That species differs in producing black instead of pale conidiomata, cylindrical to ellipsoidal conidia, a less developed textura prismatica between the covering and basal stroma, and its habit on *Camellia sinensis* (L.) Kuntze. The herbarium specimen W 618 (labelled by Petrak as "*Lophodermium smilacinum* Petr. n. sp.", but never described), with a structure typical of *Terriera*, is somewhat similar to *T. petrakii* but occurs on twigs, has narrower and more confluent ascomata, and lacks an anamorph.

Ascomata of *Terriera petrakii* often occur together with *Hypoderma smilacicola* C.L. Hou & M. Piepenbr. and *Coccomyces occultus* Y.L. Lin & Z.Z. Li (Hou & Piepenbring 2006, Lin et al. 1999).

Acknowledgments

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Gloeocystidiellum kenyense in Azores and Madeira

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Abstract — The aim of this study was to clarify the identity of several collections of *Gloeocystidiellum* from Azores Archipelago and Madeira Island that were initially related to the *Gloeocystidiellum porosum–clavuligerum* group. Three new ITS nrDNA sequences were aligned and analysed phylogenetically with 18 homologous sequences from GenBank. The two main lineages, with very high bootstrap value, that were detected correspond to two well-separated species, *G. kenyense* and G. *clavuligerum*. The sequences obtained from Azores and Madeira specimens clustered in the *G. kenyense* clade. *Gloeocystidiellum kenyense* is described and illustrated from the specimens collected in Macaronesian region, and its affinities with *G. clavuligerum* are discussed.

Key words — *Basidiomycota*, *Gloeocystidiellaceae*, corticioid fungi, oceanic islands, taxonomy

Introduction

In the framework of our studies about corticioid fungi from oceanic islands (Dueñas et al. 2008a, b, Melo et al. 2008, Telleria et al. 2008, 2009a, b, c), we found several collections of a *Gloeocystidiellum* species. These collections from Azores Archipelago and Madeira Island are related, according to Larsson & Hallenberg (2001), to the *Gloeocystidiellum porosum–clavuligerum* group.

Donk (1931) described *Gloeocystidiellum* (*Russulales, Gloeocystidiellaceae*), selecting *G. porosum* (Berk. & M.A. Curtis) Donk as type, to accommodate the corticioid fungi with monomitic hyphal system, gloeocystidia, and smooth or ornamented amyloid spores. Larsson & Larsson (2003) accept *Gloeocystidiellum*

in a strict sense to include a few species with resupinate basidiomes with smooth hymenophores, monomitic hyphal systems, hyphae with clamps, SA+ or SA– gloeocystidia, and ellipsoid, ornamented and amyloid spores.

Several morphological and molecular studies as well as cultural studies and intercompatibility tests have analyzed species delimitation in the *Gloeocystidiellum porosum–clavuligerum* group (Nakasone 1982, Hallenberg 1984, 1988, Boidin et al. 1997, Larsson & Hallenberg 2001, Larsson & Larsson 2002, 2003). ITS2 and LSU nrDNA phylogenetic analyses were congruent with intercompatibility tests and gave higher resolution in differentiating this species complex than could be found in morphologically based analyses. Larsson & Larsson (2002) and Larsson & Hallenberg (2001) found that *G. porosum* and *G. clavuligerum* (Höhn. & Litsch.) Nakasone are phylogenetically widely separated and that *G. clavuligerum* should be divided into two taxa, *G. clavuligerum* and *G. kenyense*.

Our goal was to clarify the identity of *Gloeocystidiellum* specimens from Azores and Madeira mentioned above. ITS nrDNA sequences from three collections were compared with homologous sequences from GenBank published in Larsson & Hallenberg (2001) and Larsson & Larsson (2003). As mentioned in Seifert (2009), the ITS region has became the default marker for species identification in fungi. Morphological characters were compared with the taxa described in Larsson & Larsson (2002).

Materials & methods

Morphological studies

Colors of basidioma are according to ISCC_NBS Centroid Color Charts. Dried specimens were used for light microscope study. Measurements and drawing were made from microscopic sections mounted in 3% KOH solution or Melzer's reagent and examined at up to 1250× with an Olympus BX51 microscope provided with a drawing tube. Lengths and widths of 30 spores, 10 basidia, and 10 cystidia were measured from each sample. Voucher specimens have been deposited in TFC, MA, LISU, O.

DNA extractions and sequencing

Genomic DNA was isolated from herbarium specimens using an EZNA Fungal DNA MiniPrep kit (Omega Biotek, Doraville, Georgia, USA) or a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following instructions of the manufacturers. Lysis buffer incubation was done overnight at 55°C. The primer pair ITS1F and ITS4 was used to obtain amplifications of both ITS regions, including the 5.8S of the ribosomal RNA gene cluster and small flanking parts of the SSU and LSU genes (White et al. 1990, Gardes & Bruns 1993). Amplifications were done using illustra™ PuReTaq™ Ready-To-Go™ PCR beads (GE Healthcare, Buckinghamshire, UK) as described in Winka et al. (1998) following thermal cycling conditions in Martín & Winka (2000). Negative controls lacking fungal DNA were run for each experiment to check for contamination of reagents. Results of amplifications were assayed from 5 µl aliquots

by gel electrophoresis of 2% Pronadisa D-1 Agarose (Lab. Conda, Spain). When the primer pair ITS1F/ITS4 failed, the ITS regions were amplified in two parts as mentioned in Telleria et al. (2010). Prior to sequencing the amplification products were cleaned using a QIAquick gel PCR purification kit (QIAGEN, Valencia, California, USA). Both strands were sequenced separately using primers mentioned above at Secugen S.L. (Madrid, Spain). When products were faintly visualized on agarose gels (less that 20 ng/μl), cloning was conducted with pGEM®-T Easy Vector System II cloning kit (Promega, Madison, Wisconsin, USA). From each cloning reaction, three clones were selected for sequencing, using vector specific primers T7 and SP6 at Macrogen (Korea). Sequences were edited and assembled using Sequencher™ version 4.2 (Gene Codes Corporations, Ann Arbor, Michigan, USA). BLASTN searches with MEGABLAST option were used to compare the sequences obtained against the sequences in the National Center of Biotechnology Information (NCBI) nucleotide database. The consensus sequences have been lodged in the EMBL-EBI database.

Sequences were manually aligned using Se-Al v2.0a11 Carbon (Rambaut 2002) for multiple sequences and compared with homologous sequences of *Gloeocystidiellum* spp. retrieved from the EMBL Nucleotide Sequence Databases. The alignment was analyzed using the program PAUP*Version 4.0b10 for Macintosh (Swofford 2003). The maximum parsimony analysis (MP) was inferred using the heuristic search option in PAUP. Gaps were treated as missing data. Branch lengths equal to zero were collapsed to polytomies. Nonparametric bootstrap (BP) support (Felsenstein 1985) for each clade was tested based on 10,000 replicates, using the fast-step option.

Results & discussion

From direct PCR with pair primer ITS1F/ITS4, only collections TFC 15278 (GenBank FR878082), TFC 15309 (GenBank FR878083) and MA-Fungi 80408 (GenBank FR878084) were amplified and sequenced directly.

The new sequences included the last 5 bp of 18S nrDNA, the complete ITS1—5.8S—ITS2 sequence, and the first 46 or 50 bp of the 28S nrDNA.

The BLAST search of the three new direct sequences showed an e-value of 0.0 and a maximum similarity of 99.0% with *G. clavuligerum* sequences (AF310080, AF310077, AF310079, AF310084, AF310082; AF310083) and *G. purpureum* Sheng H. Wu (AF441338). Those GenBank sequences lacked the ITS1 region and the first part of 5.8S nrDNA, thus restricting the BLAST search to the last 369 bp at the 3′ end.

The other *G. clavuligerum* sequences from Larsson & Hallenberg (2001) and Larsson & Larsson (2003) as well as the two outgroup *G. porosum* sequences (AF310101 and AF31009) were included in full length in the alignment, which contained 21 sequences and 388 positions, of which 293 were constant, 13 parsimony-uninformative, and 82 parsimony-informative. The 100 most parsimonious trees were obtained using the heuristic search option (tree length = 114; CI = 0.9211; HI = 0.0789, RI = 0.9726). The bootstrap 50% majority-rule consensus tree is shown in FIG. 1.

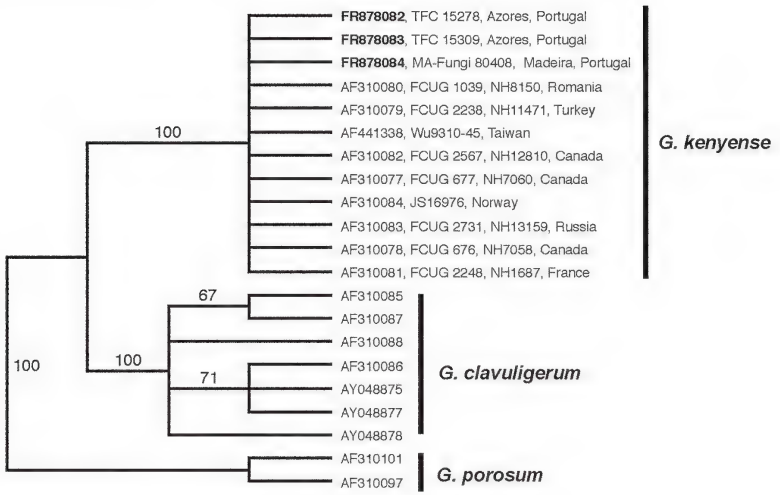

FIGURE. 1. Strict consensus tree of 100 ITS trees obtained from heuristic search in the parsimony analyses for 21 *Gloeocystidiellum* collections. Bootstrap support values (10,000) above 50% are indicated above branches. The three new sequences generated in this study are labeled with the accession number in bold.

Using *G. porosum* as outgroup, two main clades were detected, both with very high bootstrap values (100%). According to Larsson & Hallenberg (2001) and Larsson & Larsson (2002, 2003), the clades correspond with two well-distinguished species, *G. kenyense* and *G. clavuligerum*. As shown in FIG. 1, the three Azores and Madeira sequences cluster in the *G. kenyense* clade. Comparison of our collections with the *G. kenyense* holotype (L. Ryvarden 8996, herb. O) and protologue description (Hjortstam 1987) revealed no morphological differences.

Gloeocystidiellum kenyense Hjortstam, Mycotaxon 28: 29. 1987 FIG. 2

The Azores and Madeira specimens have resupinate basidiomata that are closely adnate, ceraceous, thick and hard with a smooth cream to ochraceous (89. p. Y–70.l.OY) hymenophore that is cracked when dried and an undifferentiated margin. The hyphal system is monomitic with densely interwoven, richly branched, thin-walled hyphae and clamped at all septa. Gloeocystidia are variable, originate from all levels, 35–85 × 7–16 µm, often with moniliform apical appendices, SA–. Basidia are cylindrical, (13–)21–27 × 4–5 µm, with four sterigmata. Basidiospores are subglobose to ellipsoid, 3.5–5 × 3–3.5 µm [L = 4.1, W = 3, L/W = 1.36], amyloid, ornamented.

MATERIAL EXAMINED: **PORTUGAL. AZORES**: PICO ISLAND, S. Roque, Mistério da Prainha, 38°29′42″N 28°16′01″W, 380 m a.s.l., on *Pittosporum undulatum* Vent.

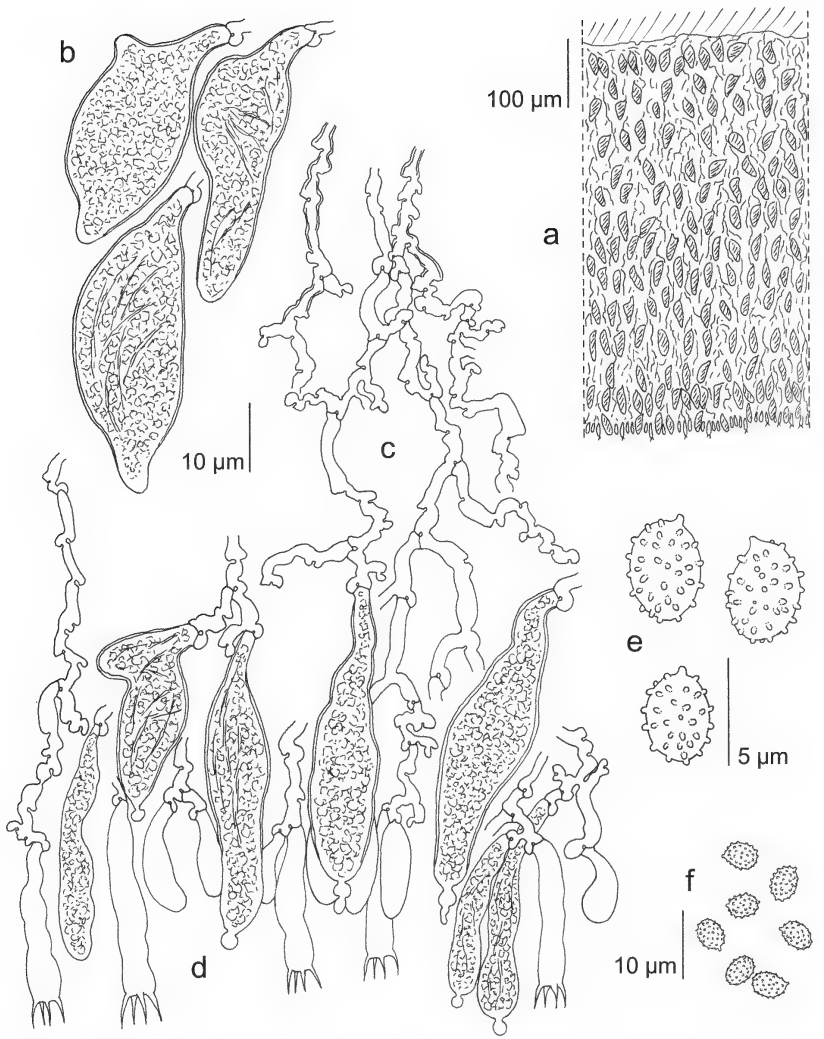

FIGURE. 2. *Gloeocystidiellum kenyense* (LISU 210747). a: section through basidiome; b: basal gloeocystidia; c: hyphae; d: hymenial layer with basidia and gloeocystidia; e, f: basidiospores.

(*Pittosporaceae*), 25 Febr. 2005, E. Beltrán-Tejera & J.L. Rodríguez-Armas (TFC 15278, GenBank FR878082); S. Roque, Meia Encosta de Santa Luzia, Travessa da Cima, 38°31′13″N 28°22′14″W, 483 m a.s.l., on *Pittosporum undulatum*, 25 Febr. 2005, E. Beltrán-Tejera & J.L. Rodríguez-Armas (TFC 15309, GenBank FR878083). MADEIRA: Porto Moniz, Seixal, Chão da Ribeira, 32°47′56″N 17°06′52″W, 485 m a.s.l., on *Ocotea*

foetens (Aiton) Benth. & Hook.f. (*Lauraceae*), 27 Jun. 2000, M.T. Telleria, 14140Tell. (MA-Fungi 80408, GenBank FR878084); 428 m a.s.l., on unidentified wood, 01 May 1998, I. Melo, 7572IM (LISU 210747). **KENYA. Eastern Prov.**: Aberdare Mounts. Kimakia Forest sta., 0°45′S 36°50′W), alt. c. 2200–2400 m a.s.l., 16–18 Jan. 1973, L. Ryvarden 8996 (holotype, O). **TANZANIA. Arusha Prov.**: Arusha Nat. Park, Mountain Meru east slope, 1800–2300 m a.s.l., 08 Febr. 1973, L. Ryvarden 10027 (O). **RÉUNION**. Cilaos, 28 March 1990, G. Gilles, Boidin 14289 (O).

According to Larsson & Larsson (2002), *G. kenyense* and *G. clavuligerum* differ in their spore sizes, as well as in gloeocystidial contents, which are SA– and SA+ respectively.

Gloeocystidiellum kenyense is widely distributed and known from Europe, North America, Africa, and Taiwan on decaying angiosperm wood (Larsson & Larsson 2002). In the Azores Archipelago it was found on *Pittosporum undulatum*, an introduced evergreen tree native to SE Australia that today dominates most of the low altitude forests of the Azores archipelago. On Madeira Island the species was found on *Ocotea foetens*, an indigenous species characteristic of the laurisilva, the Macaronesian subtropical mountain moist forest known from Madeira and Canary Islands.

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A new species of *Skeletocutis* (*Polyporaceae*) on bamboo in tropical China

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Abstract — *Skeletocutis bambusicola* sp. nov. is described and illustrated from the tropical region of Yunnan, southern China. The species is characterized by the annual, resupinate cream-colored basidiocarps, small pores, a dimitic hyphal system, small ellipsoid basidiospores, and habit on dead bamboo. The combination of characters distinguishes *S. bambusicola* from other known species of *Skeletocutis*.

Key words — *Polyporales*, polypore, taxonomy

Introduction

Encrustation of hyphae at the dissepiment edges of polypores is a special and steady morphological character in taxonomy. Species with encrusted hyphae have been referred to three genera: *Tyromyces* P. Karst., *Piloporia* Niemelä, and *Skeletocutis* Kotl. & Pouzar (Niemelä 1998). Niemelä (1998) differentiated *Skeletocutis* from the other two genera by its strong tendency to form effused basidiocarps with a homogeneous to duplex context and hyaline hyphae.

Niemelä (1998) reviewed the collections of resupinate *Skeletocutis* species from East Finland and North Sweden, while Dai (1998) provided a key to all accepted *Skeletocutis* species from China. More recently, Núñez & Ryvarden (2001) delimit *Skeletocutis* based on its dimitic system and encrustation of the hyphae at dissepiment edges.

Phylogenetically, *Skeletocutis* is closely related to *Tyromyces* (Kim et al. 2001) and clusters within the *Polyporales* (Miettinen & Larsson 2011).

Two species, *Skeletocutis lenis* (P. Karst.) Niemelä and *S. vulgaris* (Fr.) Niemelä & Y.C. Dai, were transferred to a new genus, *Sidera* Miettinen & K.H. Larss. (*Hymenochaetales*) based on ITS (internal transcribed spacer) and LSU (large subunit) nuclear ribosomal DNA (nrDNA) sequence analyses (Miettinen & Larsson 2011). Although representing different orders, *Skeletocutis* and

Sidera share many morphological characters, including whitish resupinate basidiocarps (in many species) with small pores, allantoid basidiospores, and narrow skeletal hyphae (Miettinen & Larsson 2011). The main difference between the two genera is that *Skeletocutis* usually has hyphae encrusted by fine crystals on the tube mouths, whereas in *Sidera* the dissepiment edge hyphae are smooth or covered with a few faceted crystal clusters (Niemelä 1998, Miettinen & Larsson 2011). Therefore species with faceted, rather than fine, crystals at the dissepiment edges should be excluded from *Skeletocutis*. Moreover, molecular data do not support the concept of *Skeletocutis* as restricted to dimitic hyphal structure but one that includes species with a monomitic hyphal system (Miettinen & Larsson 2011).

Extensive studies on Chinese wood-decaying fungi have recorded 704 polypores and 505 corticioid or hydnoid fungi for China (Dai 2011, 2012a), among which many new taxa have been described from tropical China (Cui & Dai 2008, Cui et al. 2009, 2010, Yuan & Dai 2009, Dai & Cui 2011, Dai & Li 2010, Dai 2012b, Dai et al. 2009, 2010, 2011, Zhou & Jia 2010). Nonetheless, the diversity and richness of tropical wood-decaying fungi are still not well known. During a study on polypores from tropical Yunnan, an unknown species with finely encrusted hyphae growing on dead bamboo was identified. It is described and illustrated here as *Skeletocutis bambusicola* following the generic concepts of Niemelä (1998) and Núñez & Ryvarden (2001) according to its latest delimitation (Miettinen & Larsson 2011). This represents the 22nd *Skeletocutis* species recorded in China (Dai 2012a).

Materials & methods

The studied specimens were deposited at the herbarium of the Institute of Applied Ecology, Chinese Academy of Sciences (IFP, Shenyang, China). The microscopic procedure follows Dai (2010). Sections were studied at magnifications up to ×1000 using a Nikon Eclipse 80i microscope with phase contrast illumination. The spore size variation excludes 5% of measurements (given in parentheses) from each end of the range. Abbreviations used are: IKI = Melzer's reagent, IKI– = negative in Melzer's, KOH = 5% potassium hydroxide, CB = Cotton Blue, CB– = acyanophilous, L = mean spore length (average of all spores), W = mean spore width (average of all spores), Q = variation in L/W ratios among all specimens studied, and n = number of spores measured from given number of specimens. Drawings were made with the aid of a drawing tube. Special color terms follow Petersen (1996).

Taxonomy

Skeletocutis bambusicola L.W. Zhou & W.M. Qin, **sp. nov.** Fig. 1

MycoBank MB 563066

Differs from *Skeletocutis alutacea, S. borealis, S. krawtzewii,* and *S. percandida* by its habit on dead bamboo.

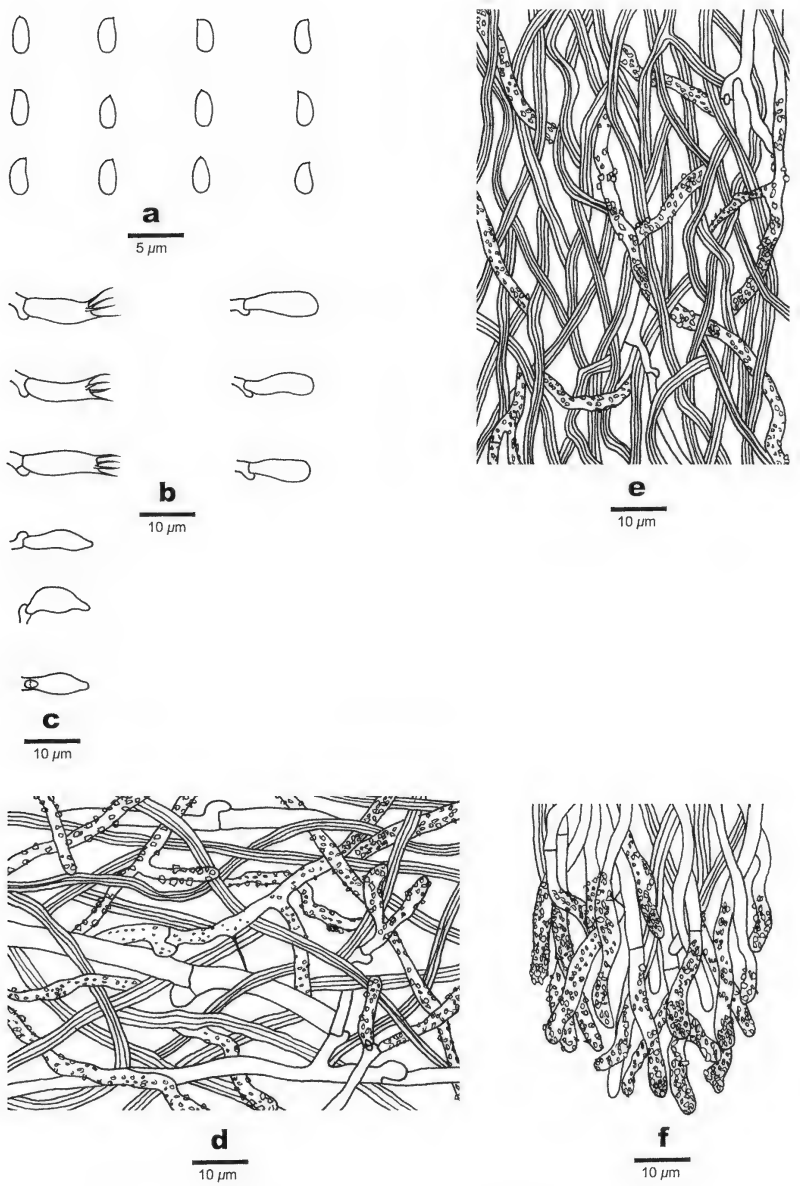

FIGURE 1. *Skeletocutis bambusicola* (holotype).
a: Spores. b: Basidia and basidioles. c: Cystidioles. d: Hyphae from subiculum.
e: Hyphae from trama. f: Hyphae at dissepiment edges.

Type: China, Yunnan Province, Xishuangbanna, Menglun County, on dead bamboo, 12.IX.2007, Yuan 3493 (**Holotype** IFP 015814).

Etymology: *bambusicola* (Lat.): refers to growth on bamboo.

Fruitbody Basidiocarps annual, resupinate, soft when fresh, becoming corky upon drying, ≤ 13 cm long, 3.5 cm wide, 1 mm thick at center. Pore surface cream when fresh, buff when dry, more or less glancing; pores round to angular, 8–11 per mm; dissepiments thin, entire to slightly lacerate. Subiculum cream and soft corky when dry, ≤ 0.2 mm thick; tubes buff-yellow, paler than pore surface, ≤ 0.8 mm long.

Hyphal structure Hyphal system dimitic; generative hyphae mostly bearing clamp connections, some at dissepiment edges with simple septa; all hyphae IKI–, CB–, unchanged in KOH.

Subiculum Hyphal structure interwoven. Generative hyphae infrequent, hyaline, thin-walled, encrusted by fine crystals, occasionally branched, winding, 1–4 µm diam; skeletal hyphae dominant, hyaline, thick-walled to distinct thick-walled with a narrow lumen, unbranched, strongly winding, occasionally bearing clamp connections, 1.5–3 µm diam.

Tubes Hyphal structure interwoven. Generative hyphae infrequent, hyaline, thin-walled, encrusted by fine crystals, occasionally branched, 1.5–3 µm in diam; skeletal hyphae dominant, hyaline, slightly thick-walled, occasionally branched, strongly winding, occasionally bearing clamp connections, 2–3 µm diam. Dissepiment edge with both generative and skeletal hyphae; generative hyphae mostly covered by fine, sharp-pointed encrustations. Hyphal pegs occasionally present. Cystidia absent, fusoid cystidioles present in the hymenium, hyaline, thin-walled, 10–17 × 3–5 µm. Basidia clavate, with four sterigmata and a basal with clamp connections, 12–20 × 4–5 µm; basidioles in shape similar to basidia, but slightly smaller.

Basidiospores Ellipsoid, hyaline, thin-walled, smooth, IKI–, CB–, (2.6–) 2.7–3.1(–3.2) × 1.5–1.9(–2.0) µm, L = 2.92 µm, W = 1.74 µm, Q = 1.66–1.70 (n = 60/2).

Type of rot White rot.

Additional specimen examined: **China, Yunnan Province**, Xishuangbanna, Menglun County, on dead bamboo, 12.IX.2007, Yuan 3500 (IFP 015815).

Remarks — *Skeletocutis bambusicola*, collected in tropical China, produces annual resupinate cream basidiocarps, finely encrusted hyphae not restricted to the dissepiment edges, and small pores and basidiospores. This combination of characters is typical for *Skeletocutis*.

The new species belongs to the thick-spored *Skeletocutis* group, which includes five other species — *S. alutacea* (J. Lowe) Jean Keller, *S. borealis* Niemelä, *S. krawtzewii* (Pilát) Kotl. & Pouzar, *S. percandida* (Malençon & Bertault) Jean Keller, and *S. perennis* Ryvarden (Niemelä 1998). *Skeletocutis*

Table 1. Comparison of six thick-spored *Skeletocutis* species based on Niemelä (1998) and specimens examined in the present study.

Species	Basidiocarp	Pores/mm	Dissepiment edge	Basidiospores [L × W]
S. alutacea	Annual, rhizomorphic	9–10	Dimitic	3.3 ×1.7 µm Q = 1.7–2.0
S. bambusicola	Annual, non-rhizomorphic	8–11	Dimitic	2.5 × 1.6 µm Q = 1.7
S. borealis	Perennial, non-rhizomorphic	(5–)6–7(–8)	Monomitic	4.0 × 1.5 µm Q = 2.5–2.8
S. krawtzewii	Annual, non-rhizomorphic	5–6	Monomitic	3.8 × 1.9 µm Q = 2.0
S. percandida	Annual, rhizomorphic	5–6	Dimitic	6.7 × 2.7 µm Q = 2.5–2.6
S. perennis	Perennial, non-rhizomorphic	(4–)5–6	Dimitic	3.1 × 1.8 µm Q = 1.7

borealis and *S. percandida* are distinguished from the new species by their cylindrical basidiospores (Niemelä 1998). The other three similar species also have ellipsoid basidiospores, *S. alutacea* is distinguished by its longer basidiospores (L = 2.9–3.8 µm, Niemelä 1998) and rhizomorphic basidiocarps while *S. krawtzewii* and *S. perennis* differ in larger basidiospores (3.4–4.1 × 1.7–2 in *S. krawtzewii;* 2.9–3.6 × 1.8–2.1 in *S. perennis*) and larger pores (5–6 per mm; Niemelä 1998). In addition, *S. krawtzewii* has monomitic hyphae at dissepiment edge and thin-walled skeletal hyphae (Niemelä 1998) and *S. perennis* has perennial basidiocarps with subparallel hyphae in the subiculum (Niemelä 1998). The main morphological differences among the six species are compared in Table 1.

Niemelä (1998) proposed that, "Ecological characteristics are important when identifying the species of *Skeletocutis*." *Skeletocutis bambusicola* was found on dead bamboo, which might be the most important evidence that separates it from other species of *Skeletocutis*.

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Paxillus albidulus, P. ammoniavirescens, and *P. validus* revisited

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Abstract—Comparison of nrITS sequences of the type specimens of *Paxillus ammoniavirescens* and *P. validus* show them to be identical. The name *Paxillus ammoniavirescens* was published in 'Spring 1999' [northern hemisphere] and that of *Paxillus validus* in August 1999, making *P. ammoniavirescens* the correct name for this species. A variant without pigments fits into *P. involutus*, suggesting that *P. albidulus* might be an albino variant of any species in the *P. involutus* complex. *Paxillus ammoniavirescens* is widespread in Europe and has been introduced in the southern hemisphere; *P. obscurosporus* is known from China and Europe; and the two taxa in the *P. involutus* clade are reported from Europe and from North America.

Keywords—*Paxillaceae*, phylogenetic species, pigment-free variants

Introduction

The genus *Paxillus* Fr. is widely distributed in the northern hemisphere, forms ectomycorrhiza with a range of host trees, is extensively used in ectomycorrhizal research (e.g. Ek 1997, Leake et al. 2001, Prendergast-Miller et al. 2011, Wilkinson et al. 2010), and is a nightmare for species recognition. There are two main species clusters — *P. rubicundulus* P.D. Orton and allies, associating with *Alnus* species, and the *P. involutus* complex, associating with a range of host trees (*Pinaceae, Fagaceae, Salicaceae*, *Corylaceae*, *Tiliaceae,* etc.). Hedh et al. (2008), who recently addressed species concepts of the European taxa in the *P. involutus* complex, recognized four species based on five gene genealogies and nrITS sequence analyses. Three of the four phylogenetic species correspond with the intercompatibility groups identified by Fries (1985). Hedh et al. (2008) recognized *Paxillus involutus* (Batsch) Fr., *P. validus*, *P. obscurosporus* C. Hahn, and a fourth species, sister to *P. involutus,* which they refrained from naming.

Table 1. Overview of *Paxillus* collections.

Species	Collection (herbarium)	Locality	Habitat	Collection date	Genbank nr ITS
ammoniavirescens	A.E. Hills 2004209 (K)	U.K.: England, Kent, Bean, Beacon Wood Country Park	in mixed forest	21 Oct. 2004	JN661711
	A.E. Hills 2005047 (K)	U.K.: England, Berkshire, Silwood Park near Ascot	with *Quercus robur*	8 Aug. 2005	JN661712
	A.E. Hills 2005059 (K)	U.K.: England, Hampshire, New Forest, Cadnam Cricket Pitch area	under *Salix caprea*	20 Sept. 2005	JN661713
	A.E. Hills 2005086 (K)	U.K.: England, Oxfordshire, Oxford, Milham Ford School	in parkland under *Tilia cordata*	30 Sept. 2005	JN661714
	A.E. Hills 2007039 (K)	France: Dept. Alpes Maritimes, Esterel-Les Espagnols	under *Alnus*	16 Oct. 2007	JN661715
	A.E. Hills 2007046 (K)	France: Dept. Alpes Maritimes, Esterel-Les Espagnols	under *Salix*	16 Oct. 2007	JN661716
	A.E. Hills 2007045 (K)	France: Dept. Alpes Maritimes, Esterel-Les Espagnols	with *Quercus suber* and *Populus*	16 Oct. 2007	JN661717
	P. Dessi (IB1997_0980, TYPE)	Italy: Sardinia, prov. Cagliari, Serramanna	—	21 Nov. 1997	JN661718
	M. Contu (UC)	Italy: Sardinia, prov. Olbia-Tempio, Olbia, Park "Fausto Noce"	near *Populus*	10 Dec. 2006	JN661719
involutus s. str. I	A.E. Hills 2005067 (K)	U.K.: England, Oxfordshire, North Leigh Common	with *Betula pendula*	24 Sept. 2009	JN661721
	E.C. Vellinga 4037 (TENN64458)	U.S.A.: TN, Great Smoky Mountains NP, Polk Co., Fork Ridge Trail	with *Picea*	3 Aug. 2009	JN661722
	M. Kuo 09130703 (UC)	U.S.A.: MI, Emmet Co., The Headlands.	—	—	JN661723
	S. Kelly (UC)	U.K.: England, Hampshire, New Forest, near Tricket Cross	—	5. Sept. 2009	JN661724
involutus II	D. Deshazer DD535 (UC)	U.S.A.: CA, Sonoma Co., Sebastopol, 406 Pleasant Hill Rd	with *Corylus* and *Quercus*	4 Dec. 2006	JN661725
	E.C. Vellinga 3211b (UC)	U.S.A.: CA, Alameda Co., Berkeley	with *Betula*	2004	JN661726
	E.C. Vellinga 3336 (UC)	U.S.A.: CA, Alameda Co., Berkeley	with *Betula*	Dec. 2005	JN661727
	E.C. Vellinga s.n. (UC)	U.S.A.: WA, King Co., Fauntleroy, Lincoln Park	with *Betula*	10 Oct. 2008	JN661728

Two other species have been described from Europe, *P. albidulus* Šutara (Šutara 1992), a predominantly white species, and *P. ammoniavirescens* (Dessì & Contu 1999), characterized by a strong green reaction of the pileus surface with ammonia. As these species were not included in the study by Hedh et al. (2008), their identity and relationship to the phylogenetic species remained unresolved.

Here, we investigate the identity of *P. ammoniavirescens* and the phylogenetic placement of taxa without pigments using nrITS sequence data and phylogenetic methods.

Material & methods

MATERIAL—Type material of *P. ammoniavirescens*, additional collections of *P. ammoniavirescens* from the type locality, various *Paxillus* collections from the UK and France, and *Paxillus* collections growing with introduced *Betula* in western North America were used for analyses (TABLE I).

DNA EXTRACTION TO SEQUENCING—DNA was extracted from dried material using a Qiagen DNeasy® Blood and Tissue kit (Qiagen, Valencia, CA, USA). PCR was done under standard conditions using the following primers: ITS-1F and ITS-4 for nrITS1, 5.8S and nrITS2 (Gardes & Bruns 1993). PCR reactions were performed with an MJ PTC-100™ thermocycler (Applied Biosystems, Foster City, CA, USA) or an Eppendorf Mastercycler® gradient (Eppendorf, North America, Inc.,Westbury, NY, USA). PCR products were cleaned using 0.5 µl of ExoSAP IT (USB Corp, Cleveland, OH, USA) per reaction and cycled at 37°C for 45 min, followed by 80°C for 15 min. Sequencing was performed using Big Dye chemistry, with the same primers as for PCR, and an ABI PRISM 3100 Genetic Analyzer (both from Applied Biosystems, Foster City, CA, USA). Sequences were edited and contigs assembled using Sequencher 4.2.2 (Gene Codes Corporation, Ann Arbor, MI, USA). All newly produced sequences were deposited in GenBank (see TABLE I).

PHYLOGENETIC METHODS—nrITS sequences of the European species in the *P. involutus* species complex available in GenBank and the UNITE database, from vouchered specimens and from environmental samples, were downloaded and added to the database. The sequences were aligned with the program MAFFT version 6 (Katoh et al. 2002; Katoh & Toh 2008), using default settings. The database was analyzed by a maximum likelihood (ML) method using RAxML version 7.2.3 (Stamatakis et al. 2008). *Paxillus rubicundulus* was chosen as outgroup.

Results

The four phylogenetic European species recognized by Hedh et al. (2008) are also recovered in our nrITS sequence analyses (FIG. 1), with high bootstrap support for *P. validus/P. ammoniavirescens* (95%), *P. involutus* I (100%), and *P. involutus* II (100%). The type collection of *P. ammoniavirescens* and the type collection of *P. validus* have identical ITS sequences. Collections from England

and France with sequences newly generated for this study also belong to *P. ammoniavirescens/P. validus*, plus one collection from New Zealand. Sister to the *P. ammoniavirescens/P. validus* clade is *P. obscurosporus*, with collections from all over Europe, and one from China. The other two taxa represent *P. involutus* clade I, with specimens in Europe and North America (Wisconsin, Tennessee, British Columbia, Alaska), and *P. involutus* clade II, with representatives in Europe and in western North America where they grow with introduced *Betula*. *Paxillus involutus* I includes a specimen from England that lacks pigments.

Discussion

Hedh et al. (2008) used five genes, ACTA, GPIA, HYDA, RABA, and SS-TUBA, for phylogenetic species recognition in the *P. involutus* group, but concluded that nrITS on its own discriminated the four phylogenetic species recovered from the multi-gene analyses. Hence, for this study, nrITS was used to assign specimens to species. However, nrITS sequences alone are not sufficient to differentiate the North American species *P. vernalis* Watling from *P. validus* (Hedh et al. 2008), nor western North American collections that are very close to *P. obscurosporus* and grow with native and introduced tree species in native and anthropogenic habitats (data not shown).

The nrITS sequences of the type collections of *P. ammoniavirescens* (Genbank JN661718) and *P. validus* (Genbank EU084666) are identical. The two original descriptions emphasize different characters, e.g. Hahn & Agerer (1999) described the relatively big crystals on the rhizomorphs of *P. validus*, whereas Dessì & Contu (1999) did not comment on this character. The reactions of the various parts of the basidiocarps with a set of chemicals is given in both articles, with slight differences. Dessì & Contu (1999) emphasized the green reaction of the pileipellis in ammonia, whereas Hahn & Agerer described this reaction as 'schwarz überhaucht;' this difference in reaction might be caused by differences in age of the basidiocarp or by different concentrations of the reagent, but neither set of authors provided that information. For KOH different concentrations were used: 15% by Hahn & Agerer (1999), 5% by Dessì & Contu (1999). In both descriptions the pileus is introduced as ribbed, the two species are similar in general stature, and the spore sizes and spore print colour of the two are comparable. Thus we conclude here, based on the molecular evidence, that the two taxa are conspecific. The next question is which name was validly published first. Both names were published in 1999. *Paxillus validus*

FIG. 1. nrITS phylogeny of European *Paxillus* species inferred by maximum likelihood (ML) analysis. Numbers at internodes refer to confidence estimates based on 100 rapid ML bootstraps. Type collections are in bold; * indicates newly created sequences and '*white' indicates the variant without pigment in the pileus. *Paxillus rubicundulus* is used as outgroup.

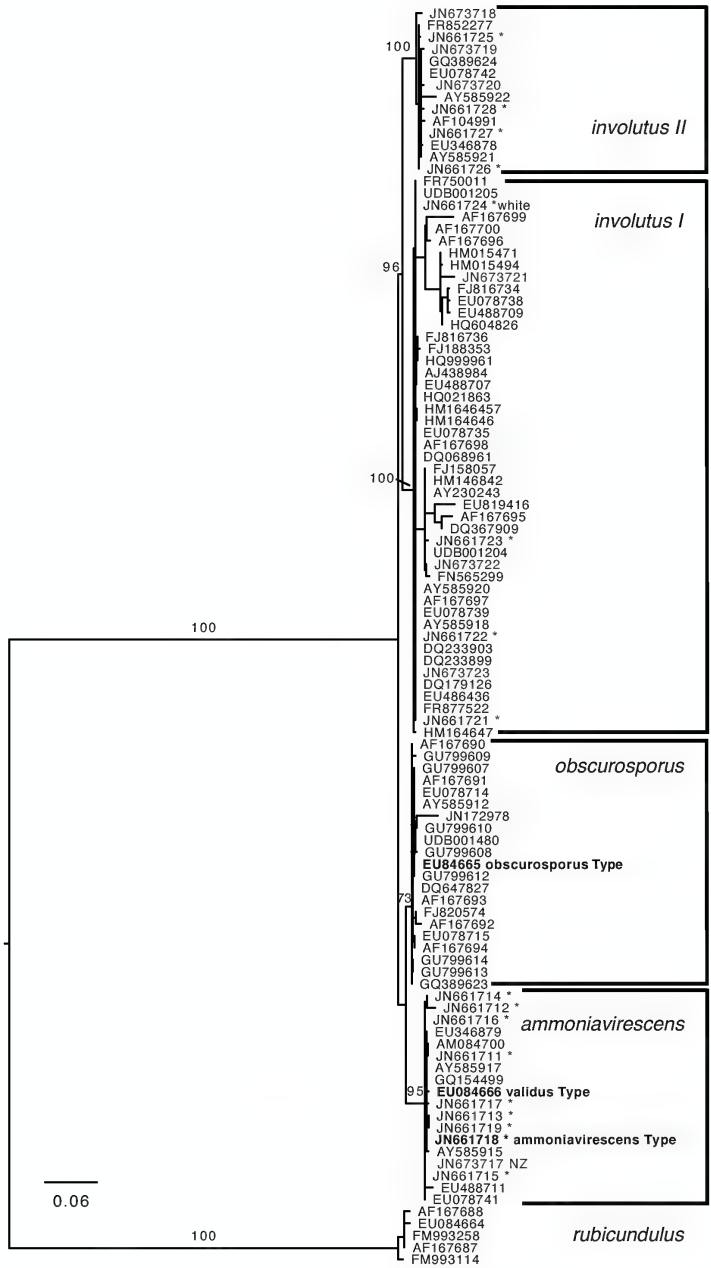
JN673718
FR852277
JN661725 *
JN673719
GQ389624
EU078742
JN673720
AY585922
JN661728 *
AF104991
JN661727 *
EU346878
AY585921
JN661726 *
involutus II
FR750011
UDB001205
JN661724 *white
AF167699
AF167700
AF167696
HM015471
HM015494
JN673721
FJ816734
EU078738
EU488709
HQ604826
FJ816736
FJ188353
HQ999961
AJ438984
EU488707
HQ021863
HM1646457
HM164646
EU078735
AF167698
DQ068961
FJ158057
HM146842
AY230243
EU819416
AF167695
DQ367909
JN661723 *
UDB001204
JN673722
FN565299
AY585920
AF167697
EU078739
AY585918
JN661722 *
DQ233903
DQ233899
JN673723
DQ179126
EU486436
FR877522
JN661721 *
HM164647
involutus I
AF167690
GU799609
GU799607
AF167691
EU078714
AY585912
JN172978
GU799610
UDB001480
GU799608
EU84665 obscurosporus Type
GU799612
DQ647827
AF167693
FJ820574
AF167692
EU078715
AF167694
GU799614
GU799613
GQ389623
obscurosporus
JN661714 *
JN661712 *
JN661716 *
EU346879
AM084700
JN661711 *
AY585917
GQ154499
EU084666 validus Type
JN661717 *
JN661713 *
JN661719 *
JN661718 * ammoniavirescens Type
AY585915
JN673717 NZ
JN661715 *
EU488711
EU078741
ammoniavirescens
AF167688
EU084664
FM993258
AF167687
FM993114
rubicundulus
100
96
100
100
73
95
100
0.06

FIG. 2. *Paxillus involutus* s. str. White variant (coll. & photo S. Kelly).

was proposed in Nova Hedwigia 69(1–2), published in August 1999 (according to the subsequent journal issue). *Paxillus ammoniavirescens* was published in Micologia e Vegetazione Mediterranea 13(2) with the nominal date "1998," but actually printed and distributed to libraries in Spring [northern hemisphere] 1999. Micologia e Vegetazione Mediterranea 13(2) arrived at L on May 20 1999, whereas Nova Hedwigia 69(1–2) arrived on 28 October 1999. This indicates that *P. ammoniavirescens* has priority as the correct name for this taxon.

Paxillus albidulus was described as 'very similar' to *P. involutus* but distinguished from that species by the white pileus, stipe, flesh, and basal mycelium. Pigments were present in the hymenium, and the spore print was 'ochreous to pale brown' (Šutara 1992). At the time *P. albidulus* was described, *P. obscurosporus* and *P. ammoniavirescens* had not yet been recognized as separate species, but the basidiocarp stature and light spore print colour exclude the possibility that *P. albidulus* is a close relative of either of these two species. Hedh et al. (2008) were not able to get sequence data from *P. albidulus*, so its identity will remain unresolved. However, the whitish collection (FIG. 2) from the U.K., also lacking pigments in pileus and stipe, fits well into *P. involutus* I (FIG. 1). *Paxillus albidulus* was reported from a predominantly coniferous forest, and as this also fits well with *P. involutus* I, we tentatively place *P. albidulus* in

FIG. 3. *Paxillus ammoniavirescens* (coll. & photo L. Perrone)
Collection: Italy, Sardinia, prov. Olbia-Tempio P., Olbia, Park "Fausto Noce",
11 Jan. 2008, det. L. Perrone & M. Contu (herb. Perrone).

synonymy with *P. involutus*. Recently another pale *Paxillus* variant was studied that also fits into *P. involutus* s. str. (Gelardi et al. 2011); that variant, described as *P. involutus* f. *eburneus* Gelardi et al., has a yellow colouration lacking in the variant from England that we studied.

The most common name used for *Paxillus* sequences deposited in GenBank is *P. involutus*; however, in many cases this is not the correct name. The option for third-party annotations is unfortunately not available, and this case emphasizes the necessity for it (Bidartondo et al. 2008).

Most taxa are widely distributed throughout the northern hemisphere, but unfortunately there are very few data from Asia. *Paxillus involutus* I is recorded from many European countries and North America (from Alaska to Tennessee in the southeast). *Paxillus involutus* II is widespread in Europe (and possibly also in boreal North America) but is better known from introduced *Betula* planted in the urban areas of the North American western states (Washington to California); its occurrence in the Great Smoky Mountains NP (Tennessee) is also confirmed (FIG. 1). *Paxillus obscurosporus* is widespread in Europe and known from China (GenBank DQ647827, as '*P. vernalis*'). Lastly, *P. ammoniavirescens* is known from Europe and introduced into New Zealand. [Southern hemisphere *Paxillus* species have been shown to belong

to a separate lineage and family for which Bresinsky et al. (1999) erected the genus *Austropaxillus* Bresinsky & Jarosch]. Vizzini (2008) identified a Moroccan collection growing under *Eucalyptus* as *P. ammoniavirescens*. After publication of Hahn & Agerer (1999) many more distribution and habitat data have become available for *P. ammoniavirescens* and *P. obscurosporus;* Hahn & Agerer's speculations that these are introduced species in Europe no longer seem to be justified, as both species have been recovered from native habitats within Europe and records from outside Europe are scarce. Hedh et al. (2008, 2009), who recorded all four species from the same county in Sweden, have shown that strains representing all four phylogenetic species were able to form ectomycorrhizas with *Betula* and with *Picea* seedlings, but not all tested strains succeeded in forming mycorrhizas and the success rate among strains varied greatly as well. How the strains behave under natural conditions is, of course, a different question.

Taxonomy

Paxillus ammoniavirescens Contu & Dessì, in Dessì & Contu, Micol. Veg. Medit. 13(2): 123. March 1999 ["1998"]. FIG. 3

HOLOTYPE: IB, 21 Nov. 1997, P. Dessì; GenBank nrITS JN661718.

= *Paxillus validus* C. Hahn, in Hahn & Agerer, Nova Hedwigia 69(1–2): 261. August 1999.
HOLOTYPE: M, 8 Oct. 1997, C. Hahn, CH 243-97; GenBank nrITS EU84666.

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The loan of the type collection of *P. ammoniavirescens* from IB is greatly appreciated. Alan Hills was instrumental in the understanding of *P. ammoniavirescens* and we warmly thank him for sending many specimens from different habitats. We also thank Luigi Perrone for permission to use his photo of *P. ammoniavirescens*. Michael Kuo and Darvin DeShazer contributed North American specimens, and Manfred Binder and Karen Hughes allowed us to use their pre-submission nrITS sequences. Comments and suggestions by Dr. Pierre-Arthur Moreau, Prof. Roy Watling, and Dr. Shaun Pennycook were very helpful for the final version of the manuscript.

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Two new *Heteroconium* species and two other forest microfungi newly recorded from China

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Abstract — Two new species, *Heteroconium annesleae* on decaying branches of *Anneslea hainanensis* and *H. neolitseae* on decaying branches of *Neolitsea obtusifolia,* are described, illustrated, and compared with closely related taxa. Illustrations are also provided of two other anamorphic fungi, *Xenoheteroconium bicolor* and *Craspedodidymum fimbriatum*, which are recorded for the first time from China.

Key words — conidial fungi, Hainan Province, systematics

Introduction

Petrak (1949) erected *Heteroconium* with *H. citharexyli* Petr. as the type species. The genus is distinguished by euseptate, fusiform, cylindrical to oblong conidia that arise in acropetal unbranched chains from solitary conidiophores with monoblastic, terminal, determinate or percurrent conidiogenous cells (Petrak 1949, Ellis 1971, Castañeda et al. 1999, Taylor et al. 2001). Castañeda et al. (2008) provided a key to 16 species of *Heteroconium*, and 19 species are currently accepted. Few other genera produce unbranched acropetal chains of phragmoconidia (which can almost be scolecoconidial). *Lobatopedis* has branched conidiophores with lobed basal cells and rhexolytic conidial secession. *Lylea* has short inconspicuous conidiophores and symmetrical cylindrical distoseptate conidia with rounded ends and extremely thick walls. Its primary conidia may produce lateral conidia that, as in *Xenoheteroconium*, can develop from non-apical cells. *Pirozynskiella* has unbranched acropetal conidial chains but is a tropical mycoparasite of *Asterinaceae.*

Xenoheteroconium was established by Bhat et al. (Bhat & Kendrick 1993) to accommodate *X. bicolor*. The genus is unique in having a bi-coloured

conidiophore, with the darker apical portion developing lateral conidia before seceding and acting as a propagule.

Craspedodidymum Hol.-Jech. (Holubová-Jechová 1972) is typified by *C. elatum* Hol.-Jech., which has branched conidiophores terminating in apically inflated phialides with large funnel-shaped collarettes, producing pale brown aseptate conidia in slimy heads.

Decaying wood in the tropical forests of Hainan Province supports a high level of fungal diversity, and many wood-inhabiting macrofungi and microfungi have been recently discovered there (Ma et al. 2008, Dai et al. 2009, Zhang et al. 2009a,b, Dai & Li 2010). During continuing investigations of saprobic microfungi, numerous conidial species were collected on decaying branches, among which two have morphological characteristics of *Heteroconium*. They differ from any described species and are proposed as new to science. Two other fungi are recorded for the first time from China. The specimens are deposited in HSAUP (Herbarium of the Department of Plant Pathology, Shandong Agricultural University) and HMAS (Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences).

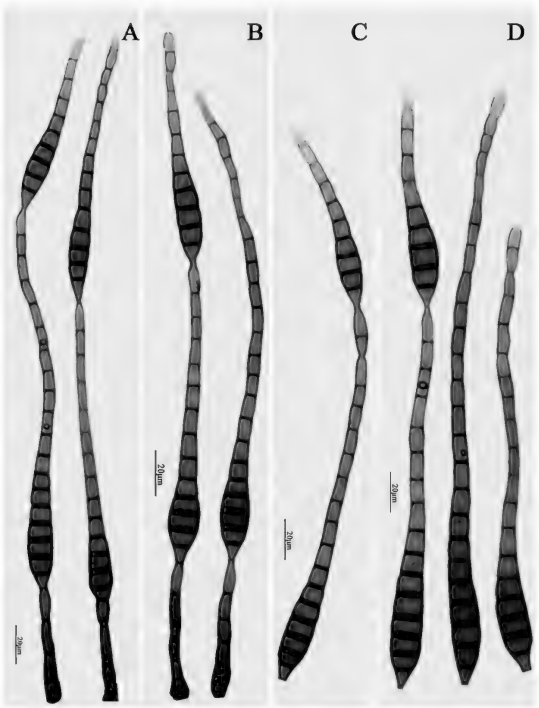

Fig. 1. *Heteroconium annesleae*.
A–B. Conidiophores, conidiogenous cells and conidia. C–D. Conidia.

Heteroconium annesleae S.C. Ren & X.G. Zhang, **sp. nov.** FIG. 1

MycoBank MB563628

Differs from *Heteroconium decorosum* in producing much larger (95–300 × 9.5–15 µm) and differently shaped conidia.

TYPE: China. Hainan Province: tropical forest of Bawangling, on decaying branches of *Anneslea hainanensis* (Kobuski) Hu (*Theaceae*), 15 May 2011, Sh.C. Ren (**holotype**, HSAUP H8492; **isotype** HMAS 146160).

ETYMOLOGY: in reference to the host genus.

COLONIES on natural substratum effuse, hairy, dark brown. Mycelium partly superficial, partly immersed, composed of septate, smooth, pale brown, branched, 2–5 µm wide hyphae. CONIDIOPHORES macronematous, mononematous, solitary, erect, unbranched, cylindrical, straight, smooth, brown, 3–5-septate, 55–65 × 4.5–8.5 µm. CONIDIOGENOUS CELLS integrated, terminal, monoblastic, cylindrical, tapered to a truncate apex, determinate, smooth, brown. Conidial secession schizolytic. CONIDIA acrogenous, holoblastic, blastocatenate, obclavate, tapering gradually towards the apex, base truncate, smooth, 9–24-euseptate, brown, apical cells pale brown, 95–300 × 9.5–15 µm.

NOTES: *Heteroconium annesleae* is similar to *H. decorosum* R.F. Castañeda et al. (Castañeda et al. 1999) in conidial morphology, but differs from the latter in conidial shape, size, and number of septa. In *H. decorosum* the conidia are navicular, broadly fusiform to obclavate, 20–30 × 3–5 µm, and 3–6-septate.

Heteroconium neolitseae S.C. Ren & X.G. Zhang, **sp. nov.** FIG. 2

MycoBank MB563630

Differs from *Heteroconium tropicale* by longer (80–95 µm) conidia with more septa (9–16) and from *H. arundicum* by narrower (4–6 µm) conidia with more septa.

TYPE: China. Hainan Province: tropical forest of Bawangling, on decaying branches of *Neolitsea obtusifolia* Merr. (*Neolitsea*). 8 May 2011, Sh.C. Ren, (**holotype** HSAUP H8375; **isotype** HMAS 146161).

ETYMOLOGY: in reference to the host genus.

COLONIES on natural substratum effuse, hairy, dark brown. Mycelium partly superficial, partly immersed, composed of branched, septate, smooth, pale brown, 2–4 µm wide hyphae. CONIDIOPHORES macronematous, mononematous, solitary, erect, unbranched, straight, smooth, brown, 9–13-septate, 80–95 × 4.5–6.5 µm. CONIDIOGENOUS CELLS integrated, terminal, monoblastic, cylindrical, tapered to a truncate apex, determinate, smooth, brown. Conidial secession schizolytic. CONIDIA acrogenous, holoblastic, blastocatenate, cylindrical or fusiform, base truncate, smooth, 9–16-euseptate, with a wide black band at each septum, brown, unevenly pigmented, pale brown ends, 50–85 × 4–6 µm.

NOTES: *Heteroconium neolitseae* is similar to *H. tropicale* R.F. Castañeda & W.B. Kendr. (Castañeda & Kendrick 1990) and *H. arundicum* Chowdhry (Chowdhry

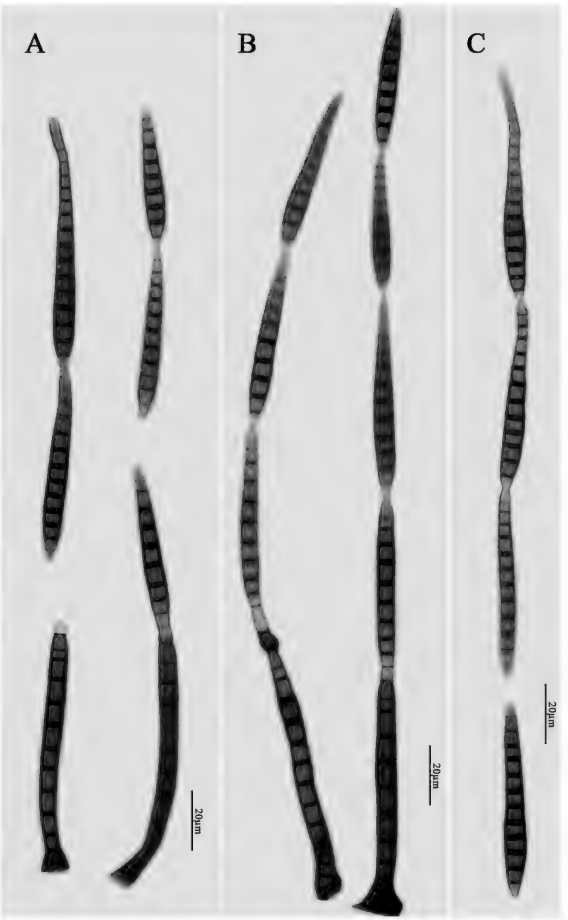

Fig. 2. *Heteroconium neolitseae.*
A–B. Conidiophores, conidiogenous cells and conidia. C. Conidia.

1980) in producing smooth cylindrical to fusiform conidia. However, *H. tropicale* is distinguished by conidia that are shorter (26–52 μm) and have fewer (3–6) septa and *H. arundicum* differs in conidia that are wider (8–12 μm) and with fewer (1–10) septa. In addition, *H. neolitseae* has a black band at each septum of conidia, a feature not found in either *H. tropicale* or *H. arundicum*.

Xenoheteroconium bicolor Bhat, W.B. Kendr. & Nag Raj, Mycotaxon 49: 81 (1993)
Fig. 3

Colonies on natural substratum effuse, dark brown, velvety. Mycelium mostly immersed in the substratum, composed of branched, septate, pale

Fig. 3. *Xenoheteroconium bicolor*.
Conidiophores, primary conidia in branched chains (indicated by white arrows) and secondary conidia (bases indicated by black arrows).

brown to brown, smooth-walled hyphae. Conidiophores macronematous, mononematous, simple, unbranched, erect, straight or slightly flexuous, cylindrical, smooth, lower part pale brown, upper part brown, with a sharp line of demarcation between the two levels of pigment, 5–7-septate, 160–195 × 3–4 μm, broadening to 5–6 μm wide at the lobed base, regenerating percurrently from broken ends. Conidiogenous cells monoblastic, integrated, terminal and intercalary in the upper part of the conidiophore axis and in primary conidia, brown, cylindrical or conical. Conidia holoblastic, phragmosporous, dry, brown; primary conidia fusiform or obclavate, straight, 2–4-euseptate, slightly constricted at the septa, 15–37 × 3.5–4.5 μm; secondary conidia cylindrical or fusiform, truncate at the base, tapering toward the tip, straight, 5–7-euseptate, slightly constricted at the septa, 35–80 × 3–4 μm.

Specimen examined: **China. Hainan Province**, tropical forest of Bawangling, on decaying branches of unidentified plant, 8 May 2011, Sh.C. Ren, HSAUP H8345; HMAS 146162.

Notes: Only one species is currently accepted in *Xenoheteroconium*. The morphology of our specimen overlaps with the type description by Bhat & Kendrick (1993).

Craspedodidymum fimbriatum Bhat & W.B. Kendr., Mycotaxon 49: 33 (1993) Fig. 4

Colonies gregarious, effuse, velvety, dark brown to black. Mycelium mostly immersed in the substratum, composed of septate, smooth, pale brown, branched hyphae. Conidiophores mononematous, fasciculate in groups, erect, straight or flexuous, unbranched, 4–8-septate, smooth, brown, paler towards the apex, percurrently regenerating, 135–210 × 4–6 μm. Conidiogenous cells monophialidic, terminal, integrated, cylindrical to clavate, slightly inflated and rounded at the apex, which bears an inconspicuous collarette, pale brown to brown. Conidia blastic-phialidic, globose, non-septate, thick-walled, pale brown, 19–22 μm diam., with numerous acellular, fibrillose, curved appendages forming a pile or coat on the surface; accumulating in colourless

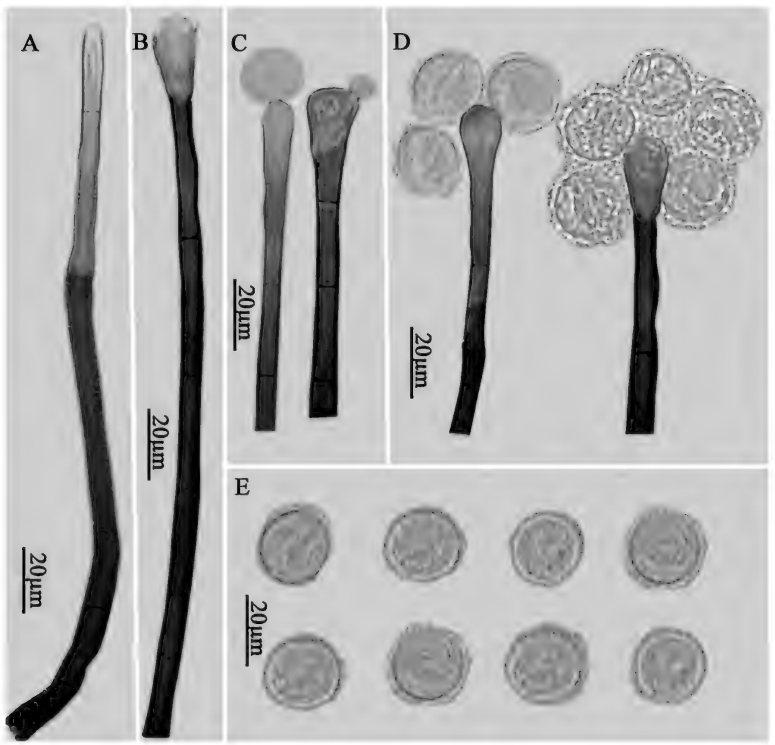

Fig. 4. *Craspedodidymum fimbriatum*. A–B. Conidiophores and conidiogenous cells. C–D. Conidiophores and conidia. E. Conidia.

slimy masses at the apex of the conidiophore, or sometimes adhering to the sides of conidiogenous cells after secession.

Specimen examined: **China. Hainan Province**, tropical forest of Bawangling, on decaying branches of unidentified plant, 8 May 2011, Sh.C. Ren, HSAUP H8405; HMAS 146164.

Notes: *Craspedodidymum fimbriatum* is unique in possessing a cylindrical to clavate conidiogenous cell, which is inflated and rounded at the apex and with an inconspicuous collarette, and slimy globose aseptate conidia with a densely fibrillose surface. The size ranges of conidia and conidiophores in our specimen overlap with those of the type specimen described by Bhat & Kendrick (1993), and other features of this taxon also match those of the original species.

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Second record of *Ramularia hypericicola* — collected in Turkey on a new host

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Abstract – *Ramularia hypericicola*, a rare anamorphic *Mycosphaerella* species, is reported and illustrated from Turkey on *Hypericum calycinum*. The Turkish collection represents the second report of this fungus, known hitherto only from the type locality.

Key words — symptoms, *Clusiaceae*, morphology

Introduction

Most species of *Ramularia* are phytopathogenic, causing leaf spots, sometimes chlorosis, or without visible symptoms, occasionally saprobic or hyperparasitic (Braun 1998). Bremer & Petrak (1947) published the first records of *Ramularia* species from Turkey, *R. anatolica* Bremer & Petr. and *R. helminthiae* Bremer & Petr. Subsequent papers about Turkish microfungi, including *Ramularia* species, were published by Bremer et al. (1948, 1952a, 1952b) and Petrak (1953, 1957). Additional Turkish records, of *Ramularia cynarae* Sacc., *R. onobrychidis* Allesch., and *R. sambucina* Sacc., were published by Karel (1958). The relatively small number (nearly 30 species) of *Ramularia* species recorded in subsequent papers (Göbelez 1964) reflects a rather poor degree of research on microfungi on plants in Turkey. However, during the past two decades research on micromycetes (including hyphomycetes) in the country has intensified (Altan & Tamer 1996, Hüseyinov & Selçuk 1999, Braun et al. 2000, Hüseyin & Selçuk 2001, Hüseyinov et al. 2002, Hüseyin et al. 2003, 2005, Melnik et al. 2004, Erdoğdu & Hüseyin 2007, Selçuk et al. 2009, 2010). We describe here one recent collection from Turkey of a *Ramularia* species previously recorded only from Russia.

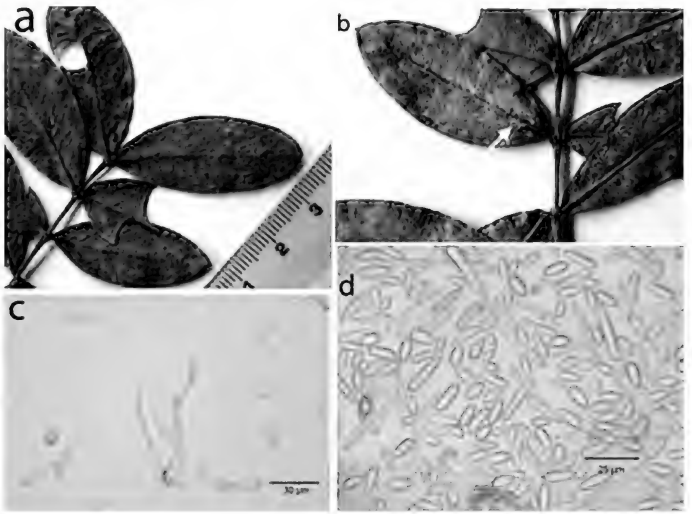

FIGURE 1. *Ramularia hypericicola* on *Hypericum calycinum*; a, spots on infected host leaves; b, punctiform grayish white caespituli; c, small fasicle of conidiophores; d, conidia.

Material & methods

The plant material was gathered from Gemlik (Bursa Province). The host plant was identified by reference to Davis (1967). Sections of the fungus were hand cut using a razor blade and microscopically examined using a Leica DM LB research microscope. The fungus was identified following Braun (1998). Author abbreviations follow Index Fungorum (2011). All examined specimens are deposited in the herbarium of Ahi Evran University, Arts and Sciences Faculty, Department of Biology, Kırsehir Province, Turkey.

Results

After careful comparison with *Ramularia* spp. described on hosts of *Hypericaceae* based on Braun (1998) the recently collected material on *Hypericum calycinum* from Turkey proved to be clearly identical with *R. hypericicola*. The following description and illustration are based on the Turkish material.

Ramularia hypericicola U.Braun, Monogr. *Cercosporella*, *Ramularia* 2: 170, 1998. FIG. 1

On both surfaces of leaves causing subcircular to irregular, occasionally angular and vein-limited, scattered sometimes confluent spots, covers more than 50% of the surface of some leaflet, firstly 0.5–1 mm across, then up to 10 mm diam. Spots on upper surface dirty brownish, dull, lover surface clearly

brown, without distinct margin. Caespituli hypophyllous, punctiform, grayish white. Mycelium internal, colourless, hyphae septate, branched, formed stromatic, colourless to yellowish-ochraceous, subglobose or slightly lengthened hyphal aggregations in leaflet tissues, 15–70(–75) µm diam. Conidiophores in small to moderately large fascicles, loose to usually dense, emerging through stomata or erumpent through the cuticle, erect, simple, straight, subcylindrical to geniculate-sinuous, continuous to sparingly septate, hyaline or subhyaline, smooth, 6–65(–70) × 2.5–5 µm. Conidiogenous cells terminal, colourless, enteroblastic. Conidiogenous scars thickened, darkened. Conidia colourless, catenate, chains occasionally branched, produced enteroblastically, single conidia smooth to faintly rough, ellipsoid-ovoid, subcylindrical, fusiform, 10–30(–35) × 4–5(–6) µm, unicellular or with a single median septa, not narrower at the septa, ends rounded or somewhat attenuated.

Specimen examined – **TURKEY, Bursa Province**, Gemlik district, Haydariye village, near Şelale, alt. 700 m, on living leaves of *Hypericum calycinum* L. (*Clusiaceae*), in hornbeam–beech (Carpineto-Fagetum) forests, 18 Nov 2010, coll. E. Hüseyin G-06.

Discussion

In 1956, the Russian mycologist M.K. Khokhryakov collected a *Hypericum* sp. with *Ramularia*-type sporulation in the Russian Far East, which he deposited in LEP as *Ramularia* sp. Braun (1998: 170) described *R. hypericicola* as a new species based on this material. *Ramularia hypericicola* is undoubtedly a rare anamorph of *Mycosphaerella*. The present record from Turkey represents the second collection of this species and the first on a known host, *Hypericum calycinum*.

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Lichenological notes 4: a revision of *Acarospora gallica* (*Acarosporaceae*)

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Abstract — *Acarospora gallica* from Europe and Asia is revised and a neotype is designated. *Acarospora hungarica* is proposed as a synonym of *A. gallica*. The anamorphic stage is reported for the first time from the species.

Key words — *Acarospora fuscata*, *Acarospora janae*, Czech Republic

Introduction

The study of the genus *Acarospora* in Europe rests on the strong foundation of the great Swedish lichenologist A.H. Magnusson, whose primary work is his classical monograph (Magnusson 1929). Nonetheless, some of the species he described are known only from the type specimens or today are poorly known by lichenologists in general. *Acarospora gallica* is among those poorly known species, mainly because of confusion with *A. fuscata*, which it can superficially resemble. It also may be a rare or infrequent species in many countries. In this paper we revise *A. gallica* as part of our study of *Acarosporaceae* in the Czech Republic. We hope this paper will lead to easier and more confident determinations of the species and to new discoveries.

Materials & methods

Specimens were studied from GZU, PRA, UPS, and the Hb. Mycologicum J. Kocourková & K. Knudsen (Hb. Myc. JK & KK). Specimens were examined using standard microscopical techniques with an Olympus SZX 7 stereomicroscope or an Olympus BX 51 fitted with Nomarski differential interference contrast. Hand-made sections were studied in water and 10% KOH [K]. Amyloid reactions were tested in Lugol's iodine [I] with and without pretreatment with K. Ascospores were measured in

water with an accuracy of 0.5 µm. Macrophotographs were taken with a digital camera Olympus DP72 mounted with QuickPhoto Camera 2.3 on the stereomicroscope. The illustrations were prepared using Adobe Photoshop.

Taxonomy

Acarospora gallica H. Magn., Kungl. Svenska Vetensk.-Acad. Handl., Ser. 3, 7(4): 282. 1929. PLATES 1–2

TYPE: France. Puy-de-Dôme: Arlane, près d.Ambert, 1900, Brevière (Hb. B. de Lesdain, Holotype, n.v., probably lost). Provence-Alpes-Côte d'Azur: Var, La Courtines près Ollivules. Sur rochers volcaniques. May, 1923, de Crozals (UPS, **Neotype designated here**).

= *Acarospora hungarica* H. Magn., Kungl. Svenska Vetensk.-Acad. Handl., Ser. 3, 7(4): 284. 1929, **syn. nov.**

TYPE: Hungary. Saro; Eperjes, 1868, Lokja (Holotype, W!).

Thallus arising from an endosubstatic hypothallus, sometimes with well-developed rhizohyphae or expanding through vegetative division. Thallus indeterminate, of dispersed or contiguous areoles, or dispersed or crowded squamules, 0.2–0.6(–1.5) mm diam., ≤ 0.2–0.5 mm thick. Areoles angular to irregular or round, broadly attached, sometimes with a single apothecium the thallus becoming reduced to a pseudothalline margin forming verrucae. Areoles becoming squamulose and lobulate in well-developed specimens, with a distinct lower surface, the elongated mycelial base broad (gomphate) or forming a stipe. Upper surface pale to dark brown, epruinose, matt. Lacking either an epinecral layer or syncortex of polysaccharides. Lower surface pale brown or white in well-developed areoles, or darkened by substrate interaction. Cortex relatively thin, 20–35 µm thick, of paraplectenchymatous cells mostly 2–4 µm diam.; upper layer brown to 10 µm thick, lower layer hyaline. Algal layer 50–100 µm, continuous, sometimes with edges of stratum uneven, algal cells mostly 10 µm diam. Medulla up to 100 µm thick, of intricate thick-walled hyphae, mostly 3–4 um thick, continuous with attaching hyphae.

Apothecia 1–12 per areole or squamule, sometimes in agglomerations; disc usually smooth, usually reddish when wetted, epruinose, 0.1–0.6 mm diam., but sometimes scabrid with ontogenic remnants of thalline plectenchyma or merging with other apothecia especially when in agglomerations. Exciple indistinct to 10–15 µm wide. Hymenium 75–100(–130) µm tall, hyaline, I+ red, blue or greenish, epihymenium 10–20 µm thick, reddish-brown. Paraphyses 1.5–2.0 µm diam., not branching, apices slightly expanded to 3 µm, sometimes wider. Asci 50–80 × 15–30, 100–200 ascospores per ascus. Ascospores hyaline, simple, mostly (2.0–)4.0–5.0(–6.5) × 1.5–2.0 µm, usually narrowly ellipsoid, but shorter ascospores broadly ellipsoid. Subhymenium usually 10–30(–50) µm tall, usually I+ blue. Hypothecium narrow, to 15 µm wide, continuous with exciple.

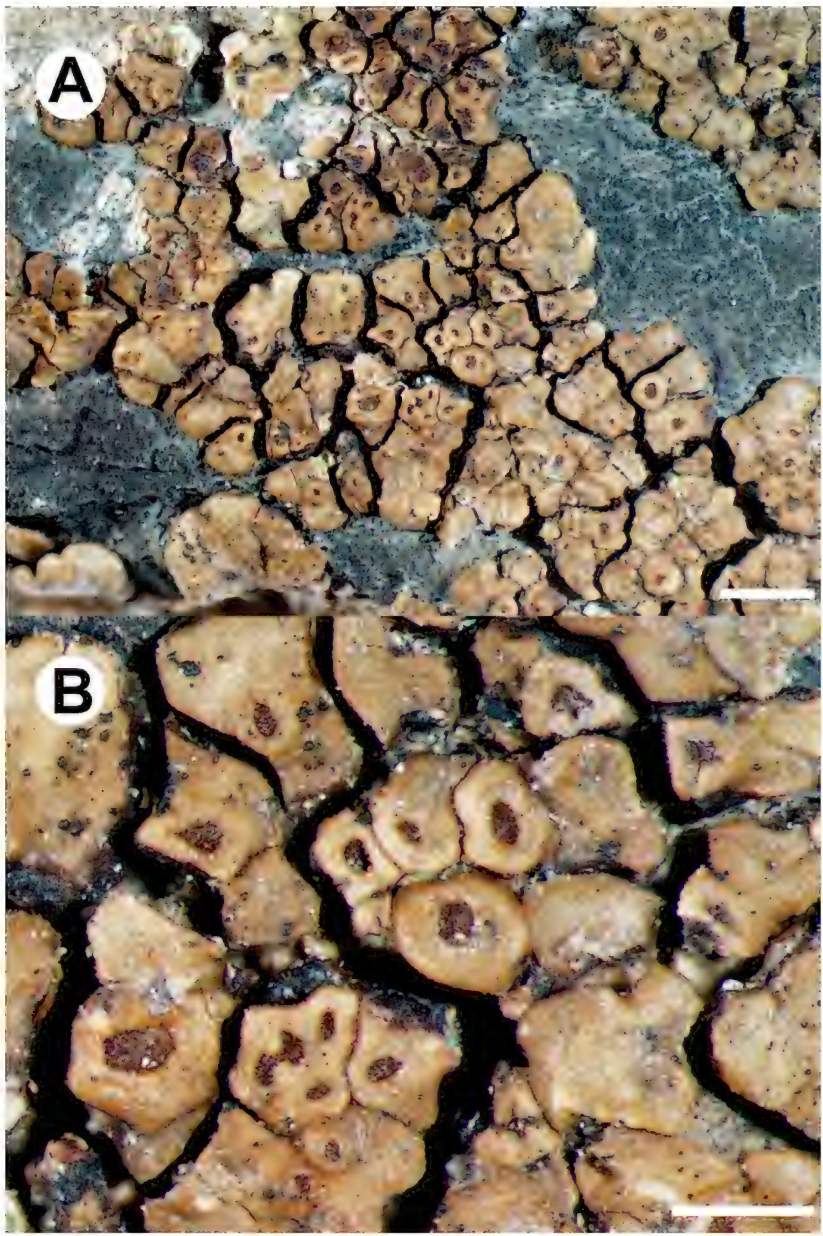

Plate 1. *Acarospora gallica* (Knudsen 12447). A, Thallus. Angular, squamulose, and lobate areoles with apothecia. B, Single and confluent apothecia. Some areoles with fungus *Lichenothelia convexa*. Scales = A = 1 mm; B = 0.5 mm.

Pycnidia usually visible as red dots, globose, to 100 µm diam., sometimes rare or absent. Conidia hyaline, simple, mostly 2.5 × 1 µm.

Chemistry— Gyrophoric/lecanoric acid, cortex and/or medulla KC+ reddish. Other spot tests negative.

Ecology & distribution— In sunny, open locations on diabase, mica-schist, sandstone, serpentine, shale, silicate-rich and volcanic rock, mostly at low (100–300 m) elevations in Asia (Siberia, Turkey) and Europe (Austria, Bulgaria, Czech Republic, Denmark, France, Germany, Hungary, Italy, Spain, Slovakia, Switzerland, Ukraine) (Magnusson 1929, 1936; John & Nimis 1998). Golubkova's reports of *Acarospora gallica* from the former Soviet Union appear to be based on Magnusson's reports, indicating that the species may be rare in the Russian Federation (Golubkova 1988). The most well developed specimens were collected in central Europe at low elevations. The highest elevation was 1820 m in Italy, collected by H. Mayrhofer (GZU).

Selected specimens examined: **Austria:** Tirol, on a roof of mica-schist, 900 m, Aug. 7, 1927, A.H. Magnusson 10954 (UPS). **Bulgaria:** Rhodope, inter Bečkovo et Staminaka, 250-330 m, ad rupes gneissaceas ad vicum publicam, Aug. 9, 1923, J. Suza s.n. (PRM 578430). **Czech Republic:** Central Bohemia, České středohoří mountains, Verneřice, a phonolit scree above Bobří soutěska pass, 420-480 m, Sept. 26, 1995, Z. Palice s.n. (PRA); Křivoklátsko Highland, distr. Rakovník, between Nezabudice and Roztoky villages, Nezabudické skály Nature Reserve, above Berounka River, 50°1'26.101"N 13°50'41.882"E, 390 m, on steep SW-facing slope, on schist outcrop in oak thin scree forest, Feb. 16, 2002, J. Kocourková 1142 (PRM 896203); Hracholusky, Čertova skála Nature Reserve, 320 m, at top of steep slope of rock outcrops, on spilite, June 28, 1996, J. Horáková 2141 (PRM 891402); ibid., 290 m, on steep slope of rocks, on spilite, July 14, 1997, J. Kocourková 3436 (PRM 906656); Pražská plošina plateau, distr. Praha-západ, Máslovice, Větrušická rokle Nature Reserve, 200 m, on SSE-facing rocky slopes, on rich shale, July 16, 2002, J. Kocourková 3310 & V. Orthová (PRM 906851); Pražská plošina plateau, Praha, Malá Ohrada, Albrechtův hill at Prokopský brook, 300 m, on diabase rock, Sept. 23, 1999, J. Kocourková 6747 (PRM 909496); Praha, Motol, natural monument "Kalvárie, Rock with cross", W-facing diabase crest with the cross on top, 50°03'96"N, 14°19'65"E, 320 m, on low diabase outcrop, Apr. 3, 2007, Z. Palice 11396 (PRA); Praha, Pitkovice, Pitkovická stráň, 50°01'26"N, 14°34'21"E, 276 m, on shale, Sept. 21, 2010, J. Kocourková 7755 & K. Knudsen 12447 (Hb. Myc. JK & KK), K. Knudsen 12457 & J. Kocourková (Hb. Myc. JK & KK, UCR). East Bohemia, the Labe valley, Chvaletice, sedimentation basin NNW of the power station, a dumped stone-terrace, ca 50°02'20"N, 15°26'40"E, 220 m, on schist, Oct. 27, 2002, Z. Palice 7956 & Z. Soldán (PRA). South Moravia, in valle fluminis Jihlavka, prope pag. Hrubšice, ad rupe arenacea, 230 m, 1919, J. Suza s.n. (UPS); Ad rupe arenacea prope Hrubšice, ca 230 m, Aug. 5, 1919, J. Suza s.n. (PRM 578429); Distr. Třebíč, Mohelno, S-SSW-facing slopes above the valley of Jihlava river, a terrace with well-lit pine-forest, 49°06'26.5"N, 16°11'08" E, 290 m, on small serpentine stone semi-immersed in the ground, Mar. 31, 2011, Z. Palice 14204 (PRA). West Bohemia, Český les, distr. Cheb, Železná hůrka, 49°59'29.5"N, 12°26'29.5"E, 575 m, on eutrophicated iron-eich phyllitic boulders, June 24, 2011, J. Halda, Z. Palice & P. Uhlík 14298 (PRA); North Bohemia: Protected Landscape Area Kokořínsko, former quarry, 300 m, Nov. 27, 1997, Z. Palice s.n. (PRA).

Plate 2. *Acarospora gallica* (Knudsen 12447).
Areoles. Stipe and well developed rhizohypha. Scale = 1 mm.

Denmark: Jylland, Schleswig-Holstein, Insel Alsen, Norburg, Sept. 11, 1913, C.F.E. Erichsen s.n. (UPS); **France:** Provence-Alpes-Côte d'Azur, Alpes-Maritimes, Nice, Peira Cava, on sandstone, Apr., 1927, J. Suza s.n. (UPS). Gallia merid., Nice, Peira Cava, ad saxa arenacea, ca 1500 m, (sepes maritim.), Apr., 1927, Suza s.n. (PRM 578431). **Italy:** Arcipelago Toscano, Prov. Livorno, Isola di Capraia, 120 m, Apr. 23, 1988, Nimis et al. s.n. (GZU). Nordl. Apennin, Prov. Pistoria: Umgebung von Abetone, Val di Luce, Alpe Tre Potenze, 1820 m, Oct. 27, 1978, H. Mayrhofer s.n. (GZU). **Russia:** Primorye, Oriente Extremo, Ditio Ussuriensis, Distr. Vladivostok, in peninsula Bassargin, Sept. 6, 1927, A. Oxner s.n. (UPS). **Slovakia:** Vihorlat, Choňkovce, andesit, 1930, J. Nádvorník s.n. (PRM 783230). **Spain:** Catalania: Vora l'osca, on volcanic rock, Sept. 1934, J. Maheu s.n. (UPS). **Switzerland:** Graubünden, Engadin, Ardez, 1400 m, Aug. 4, 1927, A.H. Magnusson 11178 (UPS). **Ukraine:** Užhorod, balvany u řeky, 1929, J. Nádvorník s.n. (PRM 783229).

Discussion

Our description of *Acarospora gallica* differs from Magnusson (1929) in understanding that the thallus is variable, from areolate to squamulose in a continuous range of developmental variation. This is probably influenced by microhabitat conditions. It is often a solitary pioneer found in sunny open sites on sharp-edged volcanic rocks, serpentine stones, diabase, shale, and even on toxic substrates in the abandoned sedimentation basins of Chvaletice and Bukovina in East Bohemia (Palice & Soldán 2004). These populations consist

usually of small broadly attached areoles, often with a single apothecium and the thallus reduced to a pseudothalline margin. On smooth substrates in microhabitats with more moisture and possibly nitrogen, particularly in central Europe, the areoles are larger, usually with multiple apothecia, and are often lobulate and squamulose, with a broad elongated mycelial base (gomphate) or a narrow stipe.

Specimens of *Acarospora gallica* with several apothecia per areole correspond to Magnusson's original concept of the species (see drawing in Magnusson 1936). *Acarospora gallica* var. *vinealis* H. Magn. has its type locality of Svätý Jur (St. Georgen) in Slovakia. The name was applied to three specimens with an even, more contiguous thallus, with areoles with multiple apothecia (Magnusson 1929). The type material of this variety was not available for study, but the description matches some specimens collected by the second author from Czech Republic. We observed that areoles with multiple apothecia were usually in the process of vegetative division or the apothecia eventually merged into a large irregularly shaped disc. Magnusson (1929) inconsistently referred thalli with predominately dispersed areoles with a single apothecium (sometimes with the thallus reduced to a pseudothalline margin) to *A. gallica* var. *devastata* (Eitner) H. Magn. Actually areoles with a single apothecium dominate in the specimens we examined, possibly because they are easier to distinguish from *A. fuscata.* We do not recognize Magnusson's two varieties as infraspecific categories but only as typological abstractions of a normal variation. *Acarospora hungarica* was described from a single specimen with well-developed ≤ 1 mm wide squamules (Magnusson 1929). We treat *A. hungarica* as a synonym of *A. gallica.*

Acarospora gallica is easily confused with *A. fuscata* (Ach.) Th. Fr., which it can resemble in color and which also produces gyrophoric/lecanoric acids. *Acarospora fuscata* has a black lower surface and is broadly attached, usually forming a contiguous areolate crust. It is the most common species throughout the range of *A. gallica*. Macromorphological characters are diagnostic for separating the two species. *Acarospora gallica* is easily distinguished from *A. fuscata* either by its lobulate squamules with pale underside or by predominance of small areoles with a single apothecium. Poorly developed specimens of *A. gallica* may be almost impossible to determine and may be reported as *A. fuscata* (as in Palice & Soldán 2004). Squamulose *A. gallica* specimens with lower sides darkened by substrate interactions can be distinguished from *A. fuscata* by the presence of a well-developed mycelial base or stipe, lobulate margins, and usually multiple apothecia per areole, sometimes growing in agglomerations. Careful examination of the thallus, may also reveal some pale lower surfaces.

Though *A. gallica* is widespread in Europe and Asia, collections are sparse, making its distribution, abundance, and conservation status uncertain for each

country where it occurs. It may be undercollected or collections may have been misdetermined as the ubiquitous *A. fuscata*. For instance, it was known in the Czech Republic from several early 20th century collections (Magnusson 1929; Suza 1928, 1931, 1947) but reported only once by Vězda (1998). But our study has established its presence in eleven localities in Bohemia and Moravia. Several vouchers were originally identified as *A. fuscata* or other species. It is frequent in Czech Republic and not threatened.

Magnusson (1929, 1930) reported *A. gallica* var. *devastata* from North America. We do not recognize *A. gallica* as occurring in North America and all the specimens from North America identified by Magnusson belong to *A. janae* K. Knudsen (Lumbsch et al. 2011; Knudsen et al. 2011). *Acarospora janae* especially differs from *A. gallica* in forming small areoles or verrucae that never become squamulose.

Galloway (2007) reported *A. gallica* from New Zealand, but we are skeptical of his determination based on the current known distribution of the species. His taxon may be conspecific with an undescribed taxon from South America collected by Adam Flakus that we are currently studying.

Another photograph of *Acarospora gallica* is available on Italic, the Information System of Italian Lichens (Nimis 2003).

Lichenothelia convexa Henssen is the only lichenicolous fungus we have collected on *Acarospora gallica* (Kocourková & Knudsen 2011). At PRM some specimens we observed were infected by *Lichenostigma gracile* Calat. et al.

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The genus *Xylaria* in the south of China – 3. *X. atroglobosa* sp. nov.

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Abstract—*Xylaria atroglobosa* (*Xylariales*, *Xylariaceae*) is described from China as a new species. It is characterized by its semi-globose stromata and ascospores with an appendage on one end. Photographs of stromata and microstructures are provided.

Key words—*Ascomycota*, pyrenomycetous fungi, taxonomy

Introduction

The southern provinces of China are characterized by a high diversity of *Xylaria* species, and many species known only from southeastern Asia have been found in southern China. *Xylaria badia* Pat., described from the Gulf of Tonkin area in Vietnam (Patouillard 1891), has been reported from Taiwan (Ju & Rogers 1999), Thailand (Thienhirun & Whalley 2004, Okane et al. 2008), Malaysia (Whalley & Whalley 2007), and China's Yunnan Province (Ma 2011). *Xylaria bambusicola* Y.M. Ju & J.D. Rogers, described from Taiwan (Ju & Rogers 1999), is found in Thailand (Okane et al. 2008) and Yunnan Province (Ma 2011). *Xylaria brunneovinosa* Y.M. Ju & H.M. Hsieh is only known from Taiwan (Ju & Hsieh 2007) and Yunnan Province (Ma 2011); *X. copelandii* Henn., restricted to *Calamus* spp. and described from Philippines (Hennings 1908), has been reported from China's Guangdong Province (Ma 2011); and *X. papulis* Lloyd was described from China (Lloyd 1921) and later found in Papua New Guinea (Van der Gucht 1995), Philippines, Taiwan (Ju & Rogers 1999), and Thailand (Okane et al. 2008). A much longer list, including two recently described new species, *X. choui* H.X. Ma et al. and *X. ficicola* H.X. Ma et al. (Ma et al. 2011a,b), would demonstrate the very unique species composition of the

genus in southeastern Asia. This paper contributes to our knowledge of *Xylaria* in the south of China.

Materials & methods

Collection, preservation, and identification methods follow Ju and Rogers (1999). Microscopic features and measurements were made from slide preparations mounted in water and Melzer's reagent. The photographs of the asci, ascal apical ring, and ascospores were taken by using a VHX-600E microscope of the Keyence Corporation. The photographs of stromatal surface were taken with a ZSA30w microscope and S70 Canon camera. The studied specimen is deposited at the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences (HKAS).

Taxonomy

Xylaria atroglobosa H.X. Ma, Lar.N. Vassiljeva & Yu Li, sp. nov. Pl. 1–6

MycoBank MB 563468

A X. fraseri *structura stromatarum et magnitudine ascosporarum, a* X. atrosphaerica *stromatis et ascosporis majoribus, ascosporae appendicula rotundata, hyalina, noncellulari praeditae, differt.*

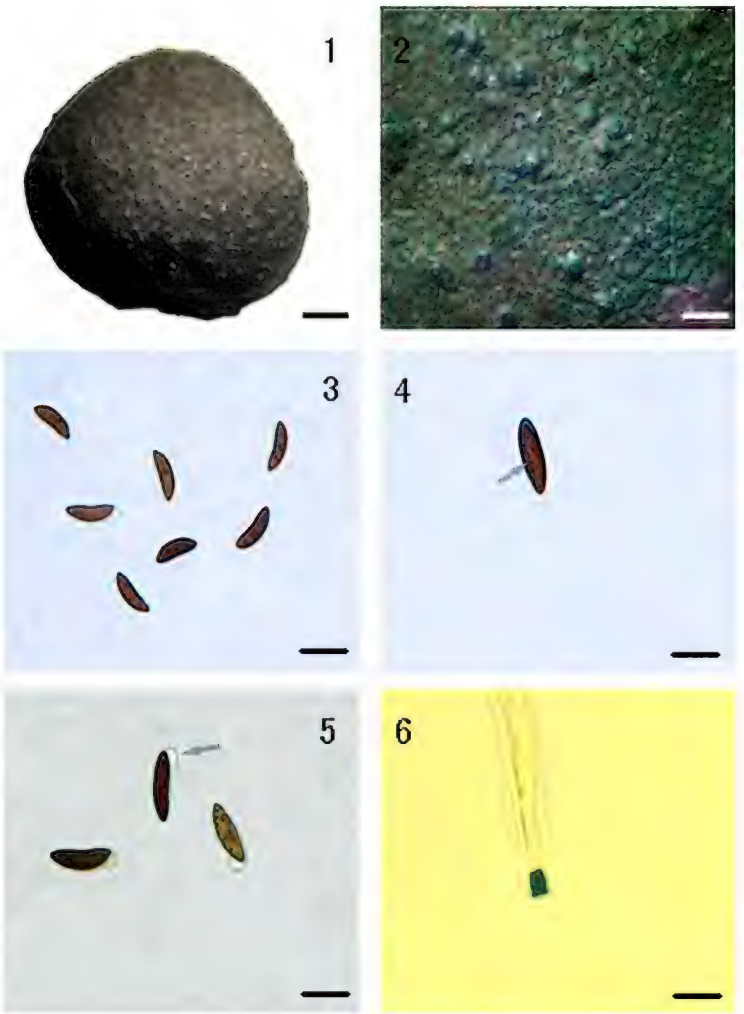
Type: China, Yunnan Province, Xishuangbanna Tropical Botanical Garden, on rotten wood, 9 Aug 1991, Peigui Liu (**Holotype**, HKAS 24003).

Etymology: Refers to the color and shape of the stroma.

Stroma hemispherical to depressed-spherical, attached to substrate with narrow connective, 3–6 mm high × 0.6–1.2 cm diam., externally black, internally white, woody, texture hard, surface smooth and perithecial mounds inconspicuous; perithecia embedded in stromata, spherical, 0.5–0.8 mm diam.; ostioles papillate. Asci 8-spored, cylindrical, long-stipitate, the spore-bearing part 150–170 μm long, with apical ring bluing in Melzer's iodine reagent, urn-shaped, 4.5–5 × 3.5–5 μm. Ascospores brown, unicellular, ellipsoid-inequilateral to crescent-shaped to navicular, with broadly to narrowly rounded ends, bearing a round hyaline noncellular appendage up to 6–7 × 3–4 μm at the one end, smooth, (24–)24.5–27(–29) × 7.5–9 μm, with oblique germ slit much less than spore-length.

Comments– *Xylaria atroglobosa* is similar to *X. fraseri* M.A. Whalley et al. (Whalley et al. 2000) in stromatal morphology and ascospore size, although its ascospores are slightly shorter. However, the stromata of *X. atroglobosa* lack white scales or a peeling layer at the surface, whereas stromata of *X. fraseri* are overlaid with a white to yellowish white peeling layer cracked into 0.2–0.4 mm broad scales. Furthermore, the ascospores of *X. fraseri* lack an appendage and their germ slit is straight.

In stromatal morphology, the new species somewhat resembles *X. atrosphaerica* (Cooke & Massee) Callan & J.D. Rogers, which can be differentiated



Figs. 1–6. *Xylaria atroglobosa*: 1—Stroma. 2—Stromatal surface. 3—Ascospores. 4—Germ slit. 5—Ascospores bearing appendage. 6—Ascal apical ring. Scale bars: 1 = 2 mm, 2 = 0.25 µm, 3 = 24 µm, 4 = 13.5 µm, 5 = 16.5 µm, 6 = 8 µm

by its smaller stromata (Callan & Rogers 1990) and smaller ascospores (18–20 × 6–7 µm) that lack an appendage.

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Moores University) for reviewing the presubmitted manuscript and giving helpful comments and suggestions. This study was supported by the National Natural Science Foundation of China (grant no. 30770005). We are also grateful to the Ministry of Agriculture of China for funding the field study in the project entitled "The Project of System Construction of Modern Agricultural Technology." We thank Dr. Pei-gui Liu (Kunming Institute of Botany, Chinese Academy of Sciences) who collected the fungus. We thank Dr. Hua Peng and Dr. Li-song Wang (Kunming Institute of Botany, Chinese Academy of Sciences), who lent us the specimen.

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A new name for a *Coltricia* (*Basidiomycota*) from India

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Abstract — Examination of the isotype of a *Coltricia* species described from India confirms it as a good morphological species. A new name, *Coltricia indica,* is proposed to replace its two previous illegitimate names. The species is characterized by very small, centrally stipitate basidiomes with shiny pilei when dried, lobed to serrate and ciliate margins, and oblong-ellipsoid, more or less thick-walled basidiospores.

Key words — *Coltricia parvula*, *Coltricia pusilla*, *Hymenochaetales*, *Hymenochaetaceae*, polypore

Introduction

Sharma & Wright (1992) described a new species *Coltricia pusilla* based on two collections of a small polypore found in two forests in Khasi Hills, Meghalaya State, India. However, their binomial was a later homonym of a name previously used by Imazeki & Kobayashi (1966) for a new species from Japan.

When J.E. Wright later became aware of Imazeki & Kobayashi's work, he proposed *Coltricia parvula* as a nom. nov. for the Indian species (Wright 1997). Unfortunately, this was also a later homonym of a combination previously published by Murrill (1904).

Our goal was to confirm the identity of Sharma & Wright's species and provide a legitimate name for it.

Materials & methods

The isotype of *Coltricia pusilla* J.R. Sharma & J.E. Wright was studied during a visit of the senior author to BAFC. Basidiomes were cut by hand for microscopic study, and sections were mounted in 3% KOH, water and Melzer's reagent (IKI). Herbaria acronyms follow Thiers (2011).

Taxonomy

Coltricia indica Baltazar, **nom. nov.** PLATE 1

MYCOBANK MB 563260

≡ *Coltricia pusilla* J.R. Sharma & J.E. Wright, Bull. Bot. Surv. India 31: 182, 1992 ["1989"], nom. illeg., non Imazeki & Kobayasi 1966.

≡ *Coltricia parvula* J.R. Sharma & J.E. Wright, in Wright, Mycotaxon 61: 419, 1997, nom. illeg., non (Klotzsch) Murrill 1904.

ETYMOLOGY: the new epithet refers to the type locality in India.

ORIGINAL DESCRIPTION (from Sharma & Wright 1992 with our additions within square brackets) — "FRUITBODY annual, centrally stipitate, coriaceous soft when fresh, hard and rigid on drying. PILEUS infundibuliform or depressed at centre, circular, 5–8 mm diam., 1–1.5 mm thick, finely depressed velutinate [to glabrous when dry], deep golden brown to cinnamon brown, shiny to glossy, narrowly concentrically zonate. MARGIN sharp, deflexed when dry, ciliate [lobed to serrate]. PORE SURFACE rusty brown, with a narrow sterile margin. PORES angular, 2–4 [2–3] per mm, entire, becoming uneven near stipe with age. TUBES ≤ 1 mm deep, light golden brown to ochraceous brown. CONTEXT ≤ 0.5 mm thick, rusty brown, fibrous. STIPE equal or tapering upwards, usually swollen at base, ≤ 2 cm × 1–1.5 mm, finely tomentose to more velutinate towards base, light rusty brown to dark brown [with a soft, loose discoid or globose base enclosing some substrate].

"HYPHAL SYSTEM monomitic, generative hyphae with simple septa, branched at broad [acute] angles, golden brown, 4–7 µm wide. SPORES oblong-ellipsoid [to ellipsoid, rarely with a tapering apex becoming almost navicular], smooth, slightly [to distinctly] thick-walled, [with a distinct apiculus,] golden brown [in KOH, lighter in water], inamyloid, [nondextrinoid,] 5–6 × 3–4.2 µm [(5–) 5.5–6(–7) × 3–4 µm]."

SPECIMEN EXAMINED: **INDIA. MEGHALAYA**, Khasi hills, Raliang forest, c. 1900 m [the protologue citation of "6500 m" is apparently an error for 6500 ft; the highest altitude in Meghalaya State is 1961 m (= 6434 ft), in the Khasi Hills], 25.IX.1986, on ground, leg. J.R. Sharma 60108 (BAFC isotype).

DISTRIBUTION: known only from two forests in the Khasi Hills, Meghalaya State, India.

Discussion

Coltricia indica is characterized by its very small, centrally stipitate basidiomes and a circular infundibuliform pileus with a lobed to serrated and ciliate margin. The stipe base is also noteworthy in that the isotype basidiomes have enlarged bases with soft and loose tissue enclosing a piece of substrate or forming a discus.

This species is very similar to *C. minor* Y.C. Dai, which also has very small basidiomes (< 0.5 cm long) and basidiospores almost identical in shape and

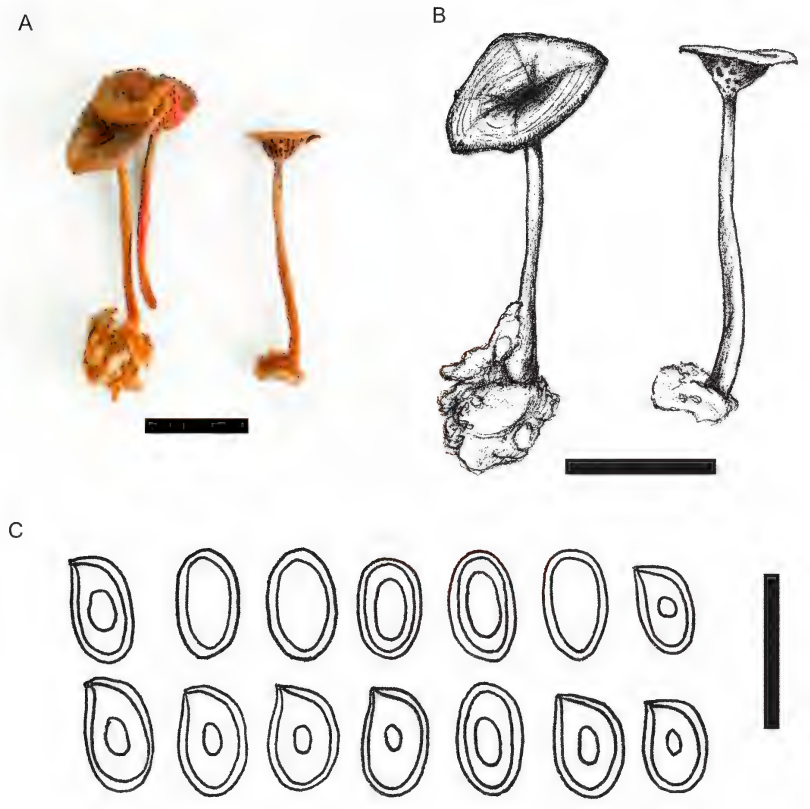

Plate 1. *Coltricia indica* (isotype).
A. Upper (left) and lateral (right) views of the pileus. B. Drawing of two basidiomes.
C. Basidiospores. Scale bars = 0.5 cm (A–B), 10 µm (C).

size. However, they differ both macroscopically and ecologically. *Coltricia minor* has laterally stipitate basidiomes, fan-shaped to spathulate pilei, non-ciliate margins, and regular stipe bases. Furthermore, *C. minor* was collected on angiosperm wood and is thus far known only from Hunan Province (China), which has a subtropical climate. *Coltricia indica*, on the other hand, was found growing on the ground of coniferous forests in India in a temperate climate due the altitude (Sharma & Wright 1992, Dai et al. 2010).

Coltricia barbata Ryvarden & de Meijer and *C. velutina* Baltazar & Gibertoni, known from Brazilian Atlantic Forest areas, also have ciliate margins and small basidiomes — pilei measuring ≤ 1.8 cm and ≤ 2.8 cm diam., respectively (Meijer 2006, Baltazar et al. 2010). However, *C. barbata* has basidiospores

that are globose to subglobose and 5–6 × 5–6 µm, compared to subglobose to broadly ellipsoid and 5.5–6.5(–7) × (4–)4.5–5.5(–6) µm in *C. velutina*. Both species also have smaller pores: 4–9 per mm in *C. barbata* and (4–)5–7 per mm in *C. velutina*.

Within *Hymenochaetaceae* Imazeki & Toki, stipitate poroid representatives with smooth basidiospores have been placed mainly in *Coltricia* Gray or *Phylloporia* Murrill. Traditionally *Phylloporia* species have a duplex pileal context with a black line below the tomentum, while *Coltricia* species have a homogeneous context and usually larger basidiospores. Ecological aspects were considered important but not decisive in circumscribing the two genera. However, recent studies have shown that some *Coltricia* species [including the type, *Coltricia perennis* (L.) Murrill] establish ectomycorrhizal associations (Tedersoo et al. 2007). Furthermore, a phylogenetic analysis by Valenzuela et al. (2011) supports *Coltricia stuckertiana* (Speg.) Rajchenb. & J.E. Wright within the *Phylloporia* clade. Except for its basidiospores, *C. stuckertiana* displays all other features typical of *Coltricia*. On the other hand, the type species of *Phylloporia* (*P. parasitica* Murrill) has not yet been included in any phylogenetic analysis. Its placement within the hymenochaetoid clade is needed for a reliable classification of the two genera, particularly as *P. parasitica* occurs in a very specialized substrate, i.e., living angiosperm leaves.

Perhaps *Coltricia* will eventually be restricted to ectomycorrhizal species. If so, we will only know the ectomycorrhizal status of *C. indica* by finding more specimens. For the time being we retain the species in *Coltricia* based on its morphology.

Based on its ornamented basidiospores, *Coltricia pusilla* Imazeki & Kobayashi is currently treated as *Coltriciella pusilla* (Imazeki & Kobayashi) Corner (Corner 1991) [≡ *Coltriciella pusilla* (Imazeki & Kobayashi) J.E. Wright, comb. superfl.]. *Coltricia parvula* (Klotzsch) Murrill is accepted as a heterotypic synonym of *Coltricia cinnamomea* (Jacq.) Murrill (Murrill 1908, Ryvarden 1976).

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MYCOTAXON

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Tuber in China: *T. microspermum* and *T. microspiculatum* spp. nov.

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Abstract — Two new species, *Tuber microspermum* and *T. microspiculatum*, are described from China. *Tuber microspermum* is characterized by small asci and spiny reticulate ascospores; *T. microspiculatum* can be separated from the other species with spinoreticulate ornamented ascospores by numerous minute meshes.

Key words — *Ascomycota*, *Tuberaceae*, truffle

Introduction

China is especially rich in species of *Tuber*. Since the first publication on this genus describing a new species from China, *T. taiyuanense* B. Liu (Liu 1985), many previously described and new species have been published (Wang 1988; Wang & Li 1991; Wang et al. 1998; Wang & He 2002; Tao et al. 1989; Chen et al. 2005; Chen & Liu 2007; Fan et al. 2011). Because all *Tuber* species grow underground, it can be very difficult to find specimens. Therefore, the number of species in China is inevitably underestimated. After continuing investigation of the genus in China, we describe and illustrate two additional new species.

Materials & methods

The materials were collected from the local mushroom market in Kunming, China. The specimens are deposited in BJTC (Herbarium Biology Department, Capital Normal University). Macroscopic characters were described from fresh specimens. Microscopic characters were described from razor-blade sections of fresh specimens mounted in 3% KOH; sections were stained with Melzer's reagent, rinsed, and mounted in polyvinyl lactic glycerol to make permanent slides for archiving with dried specimens. For scanning electron microscopy (SEM), ascospores were scraped from the dried gleba onto doubled-sided tape, which was mounted directly on an SEM stub, coated with gold-palladium, and examined and photographed with a HITACHI S-4800 SEM.

Taxonomy

Tuber microspermum L. Fan & J.Z. Cao, **sp. nov.** FIGS. 1–4

MYCOBANK MB 561881

A yellow brown truffle with small asci and small spinoreticulate ascospores that differs from *Tuber pseudoexcavatum* in an ascoma that lacks an excavated base.

TYPE: China. Yunnan Province, Kunming, from the local mushroom market. 20 Dec. 2010, Jin-zhong Cao 112 (**Holotype**, BJTC FAN149).

ETYMOLOGY: *microspermum* (Lat.), referring to the small ascospores.

ASCOMATA 1.5 cm diam., subglobose or slightly lobed, firm, solid, surface smooth to partially minutely verrucose, glabrous, yellow brown at maturity. Odor slight. PERIDIUM 200–300 µm thick, two layers; outer layer 80–120 µm thick, pseudoparenchymatous, composed of small subangular or subglobose cells mostly 7.5–12.5 µm in diam., with thickened and brown walls, darker towards the outer surface; inner layer composed of intricately interwoven hyphae, hyaline, thin-walled, branched, septate, 2.5–5 µm in diam. GLEBA brown to dark brown at maturity, marbled with numerous, narrow, branched, white to white-yellow veins. ASCI subglobose, ellipsoid or irregular, hyaline, thick-walled (3–5 µm), 45–70 × 40–60 µm, mostly with a short stalk, 1–5 spores. ASCOSPORES ellipsoid to broad ellipsoid, dark brown at maturity, 15–25(–27.5) × 12.5–20 µm in 2–5-spored asci (30–35 × 22.5–25 µm in 1-spored asci) excluding ornamentation; ornamentation distinctly spinoreticulate, spines 4–6 µm high, straight mostly, the meshes generally 3–6 across the spore width.

COMMENTS — *Tuber microspermum* is characterized by the small ascospores and asci. In ascospore pattern the new species resembles *T. pseudoexcavatum,* which is easily distinguished by a different ascoma that is typically excavated at the base (Wang et al. 1998). Other closely related *Tuber* species with spiny-reticulate spores are *T. taiyuanense* and *T. huidongense* (Liu 1985; Wang & He 2002), which can be separated from *T. microspermum* by their larger ascospores and asci.

Tuber microspiculatum L. Fan & Yu Li, **sp. nov.** FIGS 5–8

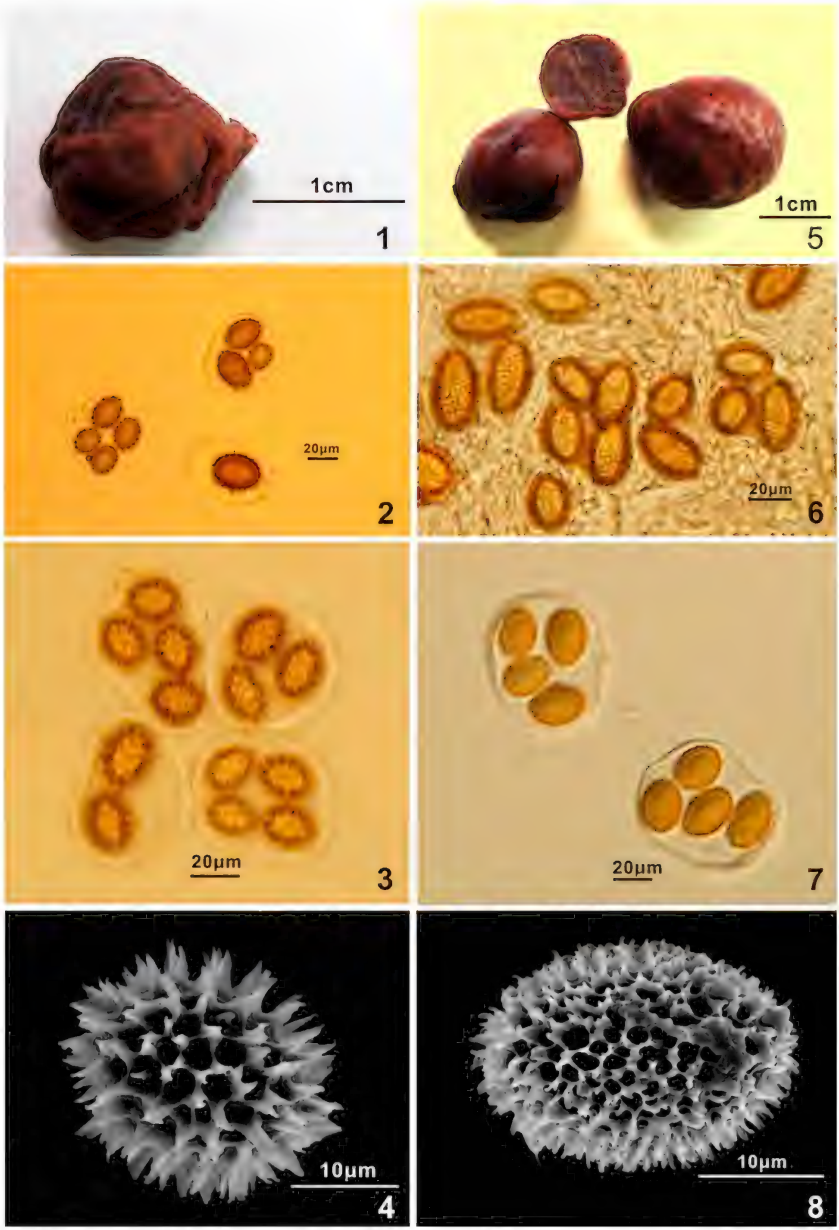
MYCOBANK MB 561882

Differs from all other *Tuber* species with spinoreticulate ascospores by an ornamentation of numerous minute narrow spine-like meshes.

TYPE: China. Kunming, Yunnan Province, from the local mushroom market. 20 Dec. 2010, Jin-zhong Cao 101 (**Holotype,** BJTC FAN138).

ETYMOLOGY: *microspiculatum* (Lat.), referring to the numerous minute meshes of the spinoreticulate spore ornamentation.

ASCOMATA 2–2.5 cm in diam., globose or subglobose, firm, solid, with or without distinct umbilicate depression at the base, surface smooth and



Figs 1–8. ***Tuber microspermum*** (BJTC FAN149, holotype): 1. Ascocarp; 2–3. Asci and ascospores observed under light microscope; 4. Ascospore observed under SEM. ***Tuber microspiculatum*** (BJTC FAN138, holotype): 5. Ascocarps; 6–7. Asci and ascospores observed under light microscope; 8. Ascospore observed under SEM.

glabrous, white yellow, pale yellow or light brown, reddish brown at maturity. Odor slight, not pungent. Peridium 200–250 μm thick, two layers; outer layer 50–100 μm, pseudoparenchymatous, composed of small subangular or subglobose cells, mostly 7.5–15 μm in diam., with slightly thickened and yellowish-brown walls, darker towards the outer surface; inner layer composed of intricately interwoven hyphae, hyaline, thin-walled, branched, septate, 2.5–5 μm in diam. Gleba brown to nearly reddish brown at maturity, marbled with numerous, narrow, branched, white-yellow veins radiating from the base. Asci subglobose, ellipsoid or irregular, 60–85 × 55–70 μm, sessile or with a short stalk, (1–)2–4 spores. Ascospores ellipsoid, a few broad ellipsoid to subglobose, light brown to yellow-brown at maturity, (20–)22.5–35(–40) × (15–)17.5–22.5 μm in 2–4-spored asci and 42.5–45 × 22.5–25 μm in 1-spored asci excluding ornamentation; ornamentation of spines 2.5–4(–5) μm high, mostly connected at bases by an alveolate reticulum, the meshes minute and numerous, generally 10–16 across the spore width, a few of large spores in 1-spored asci sometimes with 4–8 meshes across the spore width.

Comments — *Tuber microspiculatum* differs from all related species with spiny-reticulate ascospores by spore ornamentation with very minute and numerous meshes, which are so narrow that they can be easily confused with the spines under light microscope. SEM observation (Fig. 8) is helpful in clarifying the characteristics.

Species of the *T. rufum* complex, which appear similar to *T. microspiculatum* under the light microscope, can be separated by the presence of isolated spines on the ascospore surface.

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The first report of *Ceriporia lacerata* (*Phanerochaetaceae, Basidiomycota*) in Korea

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Abstract— An unrecorded *Ceriporia* species was collected during the field survey in Korea University Experimental Forests. Based on morphological characteristics and 28S, 18S, and internal transcribed spacer rDNA sequence analyses, the species was identified as *Ceriporia lacerata,* new to South Korea. A key to the Korean *Ceriporia* species is provided.

Key words— basidiomycete, polypore, taxonomy

Introduction

Ceriporia Donk is a genus of wood rotting fungi. It causes a white rot and includes species with resupinate basidiocarps with white, red, purple, orange, pink, or green pore surfaces, a monomitic hyphal system, simple septate or rarely clamped generative hyphae, lack of cystidia or other sterile hymenial elements, and subglobose to cylindric or allantoid basidiospores (Gilbertson & Ryvarden 1986).

In Korea, previously three *Ceriporia* species have been reported (Lee & Jung 2005): *Ceriporia purpurea* (Fr.) Donk, *C. reticulata* (Hoffm.) Domański, and *C. viridans* (Berk. & Broome) Donk. During our studies on diversity of indigenous fungi in Korea University Experimental Forests, two specimens of an unreported *Ceriporia* species were found. They were first identified from morphological characters, and sequence analyses of 28S, 18S and internal transcribed spacer (ITS) rDNA region supported the identification. A detailed description of the species and a key to the Korean *Ceriporia* species are provided below.

Materials & methods

Collections

The basidiocarps were collected in 2010 from one of the Korea University Experimental Forests located in Goesan-gun, Chungcheongbuk-do (36°46′13″N 127°58′57–59″E). They were dried completely overnight using an air drier at 40°C and deposited at the National Biological Resources Center (KB).

Microscopic observation

Macro- and microscopic basidiocarp features were noted from the collected specimens. Measurements and drawings were made from slide preparations mounted in 3% KOH (Largent et al. 1977) using an Olympus BX51 light microscope. Average dimensions were determined from > 20 measurements of each character. The characteristics of the four Korean *Ceriporia* listed in Table 1 are based on our observations of *C. lacerata* and descriptions of *C. purpurea*, *C. reticulata,* and *C. viridans* from previous studies (Jung 1994, Lim et al. 2000, Lee et al. 2002). A key to Korean *Ceriporia* species was constructed.

Table 1. Morphological comparison of four Korean *Ceriporia* species.

Characteristics	*C. lacerata* (this paper)	*C. purpurea* (Lim et al. 2000)	*C. reticulata* (Jung 1994)	*C. viridans* (Lee et al. 2002)
Basidiocarp	resupinate, effused, confluent	resupinate, effused, confluent	resupinate, ceraceous	resupinate, adnate
Pore surface	white, buff to ochraceous	pale to dark brownish purple	whitish to ochraceous	cream to sordid pinkish brown
Pores (per mm)	2–5	4–5	2–4	3–5
Basidia (μm)	11–16.5 × 3.5–5.5	17–21 × 5.7–6.3	15–20 × 5–6	11–15 × 4.5–6
Basidiospores (μm)	3.5–5 × 2–3	5.7–6.5 × 1.8–2.3	6–8 × 2–3	4–5 × 1.5–2

Molecular analysis

Genomic DNAs were extracted from basidiocarp hymenophores using Accuprep Genomic DNA extraction kit (Bioneer, Korea). PCR reactions were performed using Accupower PCR premix kit for 28S, 18S, and ITS rDNA, using primers LR0R/LR3 (Vilgalys & Hester, 1990), EukNS20F/EukNS1750R (Dams et al. 1988), and ITS1F (Gardes & Bruns 1993) /ITS4 (White et al. 1990), respectively. 18S PCR amplifications followed Suhara et al. (2003). 28S and ITS rDNA region conditions were as follows: initial denaturation at 95°C for 7 min, followed by 30 cycles at 95°C for 30 s, 51°C for 30 s, and 72°C for 30 s, concluded by elongation at 72°C for 7 min. DNA sequencing was performed in the DNA Engine Tetrad 2 Peltier Thermal Cycler (BIO-RAD, USA) using the ABI BigDye(R) Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) with aforementioned primers and an ABI 3730XL DNA Analyzer (Applied Biosystems, USA). The sequences obtained in this study were deposited under the GenBank accession no. JN618335, JN618336, and JN641758–JN641761. Maximum parsimony (MP) and neighbor joining (NJ) analyses of each region followed Jang et al. (2011).

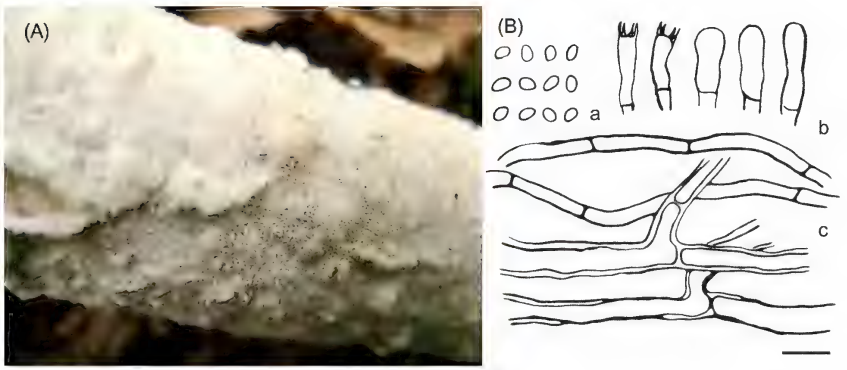

FIG. 1. *Ceriporia lacerata*. (A) Basidiocarp. (B) Microscopic features: a, basidiospores; b, basidia; c, generative hyphae. Scale bar = 10μm

Taxonomy

Ceriporia lacerata N. Maek., Suhara & R. Kondo, Mycotaxon 86: 342. 2003. FIG. 1

Basidiocarp resupinate, effused, confluent, soft when fresh, then fragile, firmly attached to the substrate; hymenophore poroid, white, buff to ochreous; dissepiments entire to lacerate; pores angular, 2–5 per mm; margin white. Hyphal system monomitic; contextual hyphae 3–5.7 μm in diameter, smooth, thin- to slightly thick-walled, simple-septate; tramal hyphae 2.3–4.6 μm in diameter, smooth, thin- to slightly thick-walled, simple-septate, cystidia lacking; basidia clavate, 11–16.5 × 3.5–5.5 μm, with 4 sterigmata; basidiospores oblong-ellipsoid to ellipsoid, 3.5–5 × 2–3 μm (ave. 3.9 ± 0.2 × 2.5 ± 0.4 μm, n = 25), smooth, thin-walled.

SPECIMENS EXAMINED: **KOREA, CHUNGCHEONGBUK-DO**, Goesan-gun, Korea University Experimental Forest, 36°46′13″N 127°58′57–59″E, fallen branches of *Quercus* sp. 07 July 2010, Yeongseon Jang (KB NIBRFG0000114705, GenBank JN618336, JN641758, JN641760; KB NIBRFG0000114712, GenBank JN618335, JN641759, JN641761).

ECOLOGY & DISTRIBUTION — China, Korea, and Japan on gymnosperms and angiosperms.

REMARKS — The characters generally match those of the holotype. However, basidiospore sizes were more variable in the Korean specimens. Suhara et al. (2003) cited 4.25–5 × 2.5–2.75 μm (ave. 4.5 ± 0.3 × 2.6 ± 0.1 μm, n = 20) for the holotype basidiospores, whereas those from our specimens measured 3.5–5 × 2–3 μm (ave. 3.9 ± 0.2 × 2.5 ± 0.4 μm, n = 25).

Phylogeny

MP and NJ analyses of the 28S, 18S, and ITS rDNA regions were conducted to confirm the morphological identification. The aligned dataset of 11 sequences

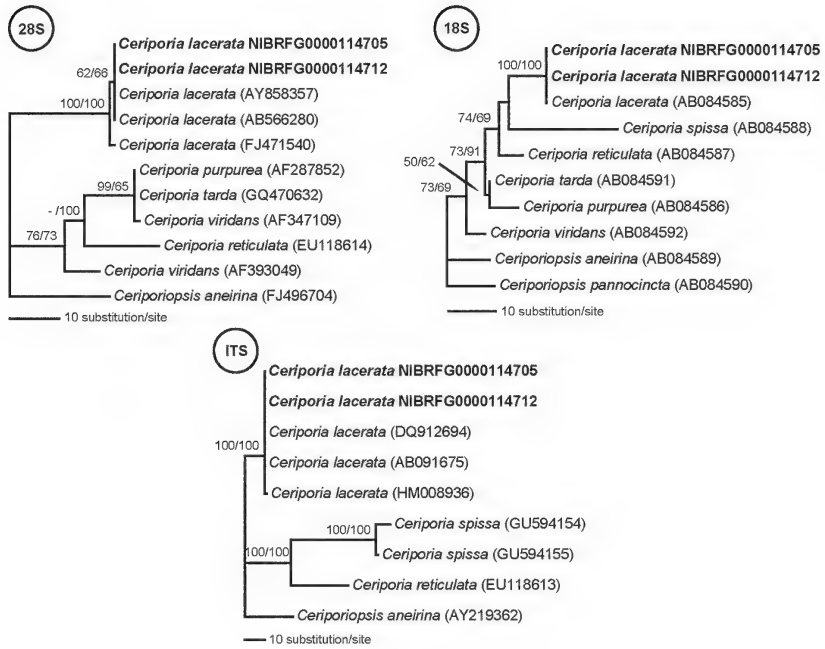

Fig. 2. 28S, 18S, and ITS rDNA region maximum parsimonious trees of *Ceriporia lacerata* and allied species. The trees were rooted by *Ceriporiopsis* species. Bootstrap values ≥ 50% are shown above branches (MP bootstrap proportions/NJ bootstrap proportions). Specimens found in this study are in bold. GenBank accession numbers in parentheses.

of 28S rDNA comprised 504 characters, of which 435 sites were constant, 24 sites variable, and 45 sites parsimony informative; tree length was 90 steps with consistency index (CI) = 0.8222 and retention index (RI) = 0.8841. The 18S rDNA dataset of 12 sequences comprised 1645 characters, of which 1578 were constant, 41 variable, and 26 parsimony informative; tree length was 79 steps with CI = 0.8608 and RI = 0.8226. The ITS rDNA dataset of 9 sequences comprised 390 characters with 281 constant, 44 variable, and 65 parsimony informative sites; tree length was 138 steps with CI = 0.9855 and RI = 0.9802. The MP and NJ analyses of all three regions clustered our sequences with previously reported sequences of *Ceriporia lacerata* with high bootstrap supports (Fig. 2). The Korean *C. lacerata* (KB NIBRFG0000114705, KB NIBRFG0000114712) showed 100% sequence similarity to a Japanese specimen (GenBank AB084585) in 18S rDNA region according to a BLAST search of GenBank. ITS rDNA region also showed 99.8% similarity (one out of 560 positions showed nucleotide difference) to a Japanese specimen (GenBank AB091675). No 28S

rDNA sequences of a Japanese specimen were available, but our specimens had 100% similarity to *C. lacerata* strains of Korea and Japan.

Discussion

Ceriporia lacerata was first described as a new species from Japan in 2003 (Suhara et al. 2003) and three years later reported from China (Cui et al. 2006, Dai 2012). In Korea, *C. lacerata* strains have been isolated from playground wood (Kim et al. 2005), CCA-treated wood (Kim et al. 2007), decayed pine tree logs (Kim et al. 2009), and decayed bamboo (Kim et al. 2011). They were also isolated from Mt. Bukhan (Seoul), Mt. Halla (Jeju), Mt. Joryeong (Chungcheongbuk-do), Mt. Mani (Incheon), Mt. Yongmun (Gyeonggi-do), and Yangpyeong Korea University Experimental Forest (Gyeonggi-do) in 2008–10 during the Korean indigenous species research project (unpublished data) and basidiocarps were found in Goesan-gun, Chungcheongbuk-do, in 2010. *Ceriporia lacerata* occurs on both angiosperm and gymnosperm wood and is widely distributed in China (Dai 2012). Our observation confirms *C. lacerata* within northeast Asia, and it is reasonable to infer that it inhabits other Asian countries in the 30–45°N latitude.

Morphologically, *Ceriporia lacerata* is similar to *C. alachuana* (Murrill) Hallenb. and *C. ferruginicincta* (Murrill) Ryvarden by having a whitish to ochreous pore surface and oblong to ellipsoid basidiospores. However, the other two species differ in having pores with entire dissepiments (3–8 per mm in *C. alachuana* and 6–8 per mm in *C. ferruginicincta*; Suhara et al. 2003), contrasting with the entire to lacerate dissepiments (2–5 per mm) of *C. lacerata.*

The 28S and ITS rDNA phylogenetic trees had similar topologies with *Ceriporia lacerata* forming a clade distinct from that of the other *Ceriporia* species (Fig. 2). On the other hand, all the analyzed *Ceriporia* species were monophyletic in 18S rDNA tree, as previously suggested by Wu et al. (2010), with *C. spissa* (Schwein.) Rajchenb. sister to *C. lacerata.* Both species occur on hardwoods and conifers, but *C. spissa* has bright orange basidiocarps (Gilbertson & Ryvarden 1986). The phylogenetic conflict represented here is probably the result of limited taxon sampling. More molecular data derived from other species are needed for the clear elucidation.

Since Kondo et al. (1999) reported that *Ceriporia lacerata* had the ability to degrade polychlorinated dibenzo-p-dioxines (PCDDs) and polychlorinated dibenzo-furans (PCDFs), its industrial potential has been investigated in pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production (Lee et al. 2007) and PAHs degradation (Lee et al. 2010). Remarkably, Long et al. (2010) reported that *C. lacerata* had the highest decolorization ability for synthetic dyes (Congo Red, Orange II, and Reactive Blue 4) among the

white rot fungal species tested in solid media. However, information regarding this species is still limited and the abilities of this species should be investigated further.

Key to the species of *Ceriporia* in Korea

1. Pore surface pale to dark brownish purple;
   (basidiospores allantoid, 5–7 μm long) ..... *C. purpurea*
1. Pore surface white to brown 2
2. Pore surface cream to sordid pinkish-brown;
   (basidiospores cylindric to allantoid, 4-5 μm long) ..... *C. viridans*
2. Pore surface white to ochraceous 3
3. Basidiospores allantoid, 6–8 × 2–3 μm ..... *C. reticulata*
3. Basidiospores oblong-ellipsoid to ellipsoid, 3.5–5 × 2–3 μm ..... *C. lacerata*

Acknowledgements

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Marasmioid and gymnopoid fungi of the Republic of Korea. 5. *Marasmius* sect. *Hygrometrici*

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Abstract — Two species of *Marasmius* sect. *Hygrometrici* from South Korea are published in this paper. Descriptions based on morphological characteristics and illustrations are provided. *Marasmius crescentiae* is a new record for the studied region, and "*M. junipericola*" represents a provisional new species.

Key words — *Fungi*, *Agaricales*, *Marasmiaceae*, taxonomy, DNA studies

Introduction

This new paper in a series on marasmioid and gymnopoid fungi collected in the Republic of Korea (South Korea, Antonín et al. 2009, 2010a,b,c, 2011) covers species of sect. *Hygrometrici*. A provisional new taxon, "*Marasmius junipericola*" is described based on its very distinct macro- and microscopic characteristics. However, we do not formally publish a new name because there was too little material for molecular sampling. *Marasmius crescentiae* is recorded as new to Korea. We provide ITS and LSU sequences of three specimens of *M. crescentiae*.

Materials & methods

Macroscopic descriptions of collected specimens are based on fresh basidiocarps collected by the first author. Colour abbreviations follow Kornerup & Wanscher (1983), herbarium abbreviations are according to Holmgren & Holmgren (1998). Authors of fungal names are cited according to the International Plant Names Index Authors website (http://www.ipni.org/ipni/authorsearchpage.do). Microscopic features are described

from dried material mounted in H_2O, KOH, Melzer's reagent, and Congo Red using an Olympus BX-50 light microscope with a magnification of 1000×. Abbreviations: L = number of entire lamellae; l = number of lamellulae tiers between each pair of entire lamellae; E = quotient of length and width in any one basidiospore; Q = mean of basidiospore E-values. Specimens are preserved in the herbarium of the Moravian Museum, Brno, Czech Republic (BRNM).

DNA extraction, PCR amplification, DNA sequencing, and phylogeny tree construction methodologies followed Antonín et al. (2010a). New GenBank accession numbers are listed in Table 1. The Bayesian-based ITS phylogeny used the general time reversible (GTR) model with gamma-distributed substitution rates (Ronquist & Huelsenbeck 2003). Markov chains were run for 1,000,000 generations, saving one tree every 100th generation and discarding the first 1000 trees. This MCMC tree was used to compute a 50% majority rule consensus of the remaining trees to obtain estimates for the posterior probabilities (PPs) of the groups. The dataset includes *M. crescentiae* and *M. micraster* (both from sect. *Hygrometrici*), with the type species of *Marasmius, M. rotula*, selected as outgroup.

Table 1. *Marasmius* specimens sequenced for this study.

Species	Specimen ID	GenBank accession number	
		ITS	LSU
Marasmius crescentiae	BRNM 714783	FJ936141	FJ936147
	BRNM 714678	FJ936142	FJ936138
	BRNM 718790	JN003840	JN003845

Results

Marasmius crescentiae Murrill, North American Fl. 9: 259, 1915. Figs 1–2

Basidiocarps single. Pileus 3–5 mm broad, broadly conical to convex with slightly depressed centre and straight margin, sometimes with slightly depressed centre (when old) centre rugulose, sulcate otherwise, margin crenulate, surface tomentose, light brown to brown (± 6–7D–E6–7, 7E6), paler (brownish orange, ± 6B–C5–6) at margin. Lamellae distant, L = 9–15, l = 0(–1), shortly to broadly adnate, slightly intervenose when old, pale cream coloured, concolorous or with coloured (± pileus colour, especially in external part), pubescent edge. Stipe 20–65 mm long, filiform, insititious, smooth, glabrous, concolorous with lamellae at apex, (reddish) black-brown (8E–F7) otherwise.

Basidiospores 6.0–9.0 × 3.0–4.5 µm, average = 7.6 × 3.7 µm, E = 1.6–3.0, Q = 1.8–2.6, ellipsoid-fusoid, fusoid or sublacrimoid, thin-walled. Basidia 17–26 × 7.0–9.0 µm, 4-spored, (broadly) clavate. Basidioles 12–23 × 4.0–9.0 µm, clavate, fusoid. Cheilocystidia of two types: [1] (10–)15–20(–23) × (4.5–) 6.0–13 µm, broom cells of the Rotalis-type, broadly clavate, pyriform, thin-walled, often with brown walls in KOH; [2] 15–30 × 4.5–9.0 µm, lageniform or fusoid, rostrate, smooth or with scattered projections at upper part of a base,

FIG. 1. *Marasmius crescentiae.*
Basidiocarps (Hongcheon, Nae-myeon, Sambong-ri, V. Antonín 08.26, BRNM 718666).

obtuse to subacute, thin-walled. PLEUROCYSTIDIA absent or rare, similar to cheilocystidia of the type 2, 17–35 × 4.5–9.0 µm, fusoid or lageniform, rostrate, thin-walled. TRAMA HYPHAE ± cylindrical, thin- to slightly thick-walled, hyaline, up to 12 µm wide. PILEIPELLIS a hymeniderm composed of (8.0–)10–21 × 7.0–14(–17) µm, clavate, pyriform or (sub) vesiculose cells of the Rotalis-type, thin-walled at base, thin- to slightly thick-walled at apex; diverticula up to 1.5×0.75 µm, numerous; thick-walled parts brown in KOH. PILEOCYSTIDIA (12–)15–25 (–30) × 4.0–8.0 µm, fusoid or lageniform, rostrate, thin- to slightly thick-walled (especially in central part), sometimes with projections in upper basal part; with chestnut brown wall in thick-walled parts. STIPITIPELLIS a cutis of cylindrical, parallel, slightly thick-walled, diverticulate, up to 5.0 µm wide hyphae with brown walls in KOH. CAULOCYSTIDIA absent. CLAMP CONNECTIONS present in all tissues.

CHEMICAL REACTIONS — No part of basidiocarps amyloid or dextrinoid.

ECOLOGY — On fallen leaves of *Acer, Quercus acutissima, Juglans,* and other broadleaved trees, dead plant stems.

SPECIMENS EXAMINED — **REPUBLIC OF KOREA**, Hongcheon, Nae-myeon, Eulsudong valley, 37°41′11″N 128°29′35″E, alt. c. 670 m, 26 July 2007 leg. V. Antonín (07.131) and R. Ryoo (BRNM 714783); Deogyusan National Park, Cheon-yeon falls, 24 Aug. 2007 leg. R. Ryoo KG 164 (BRNM 714678); Hongcheon, Nae-myeon, Sambong-ri, 26 June 2008

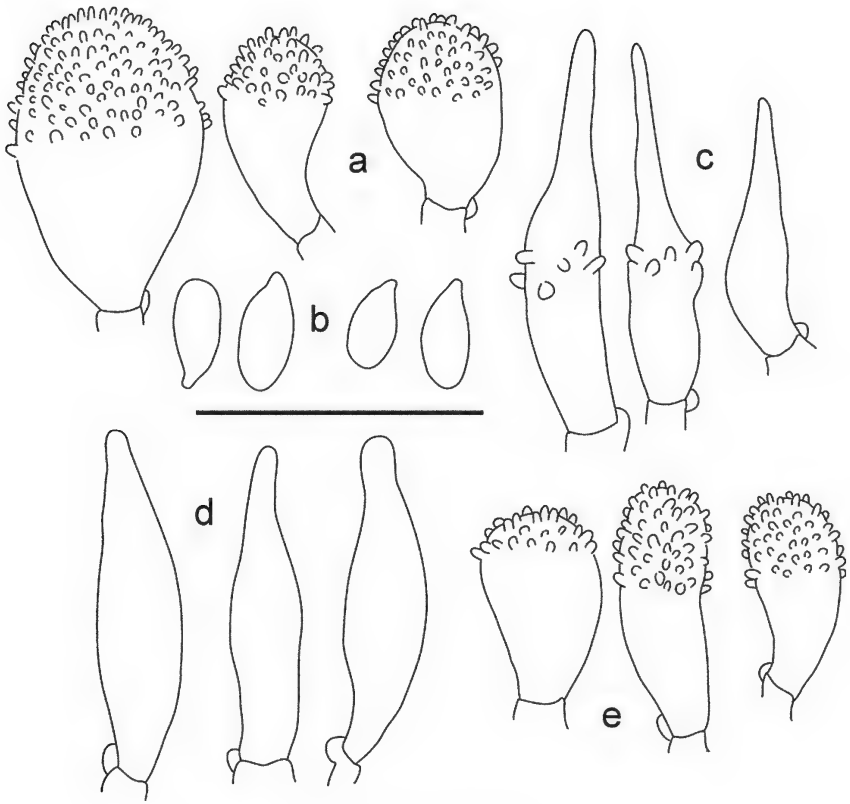

Fig. 2. *Marasmius crescentiae*.
a. Pileipellis cells, b. basidiospores, c. pileocystidia,
d. cheilocystidia (type 2) and pleurocystidia, e. cheilocystidia (type 1). Scale bar = 20 μm.

leg. V. Antonín (08.25, 08.26) and R. Ryoo (BRNM 718665, 718666); Wonju, Chiaksan National Park, between N.P. office and Guryongsa, 37°24′17″N 128°03′00″E, alt. 310–360 m, 19 July 2009 leg. V. Antonín (09.135) and R. Ryoo (BRNM 718790).

Remarks — The South Korean collections of *M. crescentiae* are characterized by small basidiocarps, a sulcate light brown to brownish orange pileus, rather large basidiospores, tetrasporic basidia, cheilocystidia of two types, absent or scattered pleurocystidia, present pileocystidia, absent caulocystidia, and present clamp connections. Although Singer (1976) observed smaller basidiospores (6.5 × 2.7 μm), Desjardin & Horak (1997) cited larger basidiospores (7.5–8.5 × 3.5–4.0 μm).

Very similar species are *M. micraster* Petch, with slightly larger basidiospores (Desjardin & Horak 1997: 9–11(–12) × (3.5–)4–5(–5.5) μm; Pegler 1986: 8.5–11 × 4.0–5.5 μm) and 18–30 μm long basidia (Singer 1976), and *M. exustus*

Berk. & M.A. Curtis, with smaller (6.5–8.0 × 4–5 µm) basidiospores (Desjardin & Horak 1997). Other species differ either by macroscopic characters (e.g., *M. corbariensis* (Roum.) Sacc.; Antonín & Noordeloos 2010) or by developed caulocystidia (e.g., *M. minutus* Peck; Antonín & Noordeloos 2010).

Three sequences obtained from collections VA 07.131, KG 164 and VA 09.135 (BRNM 714783, 714678 and 718790; Table 1) of *Marasmius crescentiae* showed a significant ITS rDNA similarity (Fig. 3). Among LSU sequences trimmed by 1167 nucleotide characters, the three sequences showed no more than five base substitutions with each other.

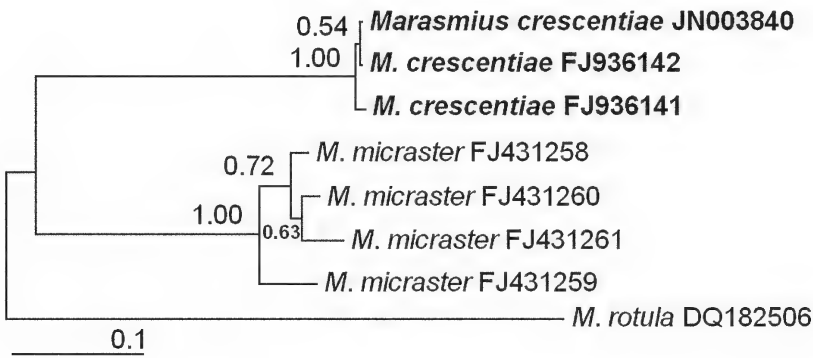

Fig. 3. Phylogenetic tree for *Marasmius* sect. *Hygrometrici* based on the ITS region (ITS1+5.8S rDNA+ITS2). Bayesian analysis running 1,000,000 generations represent mean branch lengths of a 50% majority-rule consensus tree calculated from 1000 trees revealed. Scale bar = number of nucleotide substitutions per site. **Bold face** = Species sequenced for this study.

"*Marasmius junipericola*" ad int. Fig. 4

Basidiocarps single. Pileus 1–1.5 mm broad, low convex to convex with obtusely applanate then slightly depressed centre, ± smooth when young, then slightly crenulate towards margin, covered with small brown granulose ± spiny scales, entirely brown (7E7) when young, later brown only at centre and whitish towards margin. Lamellae distant, L = 8–10 , l = 0–1, free or with small tooth, white, with concolorous, slightly pubescent edge. Stipe 4–8 mm long, filiform, insititious, entirely finely strigose-pubescent, dark brown (6–7E–F7), paler at apex.

Basidiospores (7.0–)8.0–9.5 × (4.2–)4.5–5.0 µm, average = 8.5 × 4.7 µm, E = 1.5–1.9, Q = 1.8, broadly ellipsoid, fusoid-ellipsoid, hyaline, thin-walled. Basidia 18–24 × 5.5–10 µm, 4-spored, clavate. Basidioles up to 22 × 5.0–9.0 µm, clavate, fusoid, subcylindrical. Cheilocystidia of two types, [1] 15–21 × 8.0–12 µm, broom cells of the Rotalis-type, broadly clavate, thin-walled; [2] 23–35 × 5.0–9.0 µm, lageniform or fusoid, rostrate, obtuse, thin-walled.

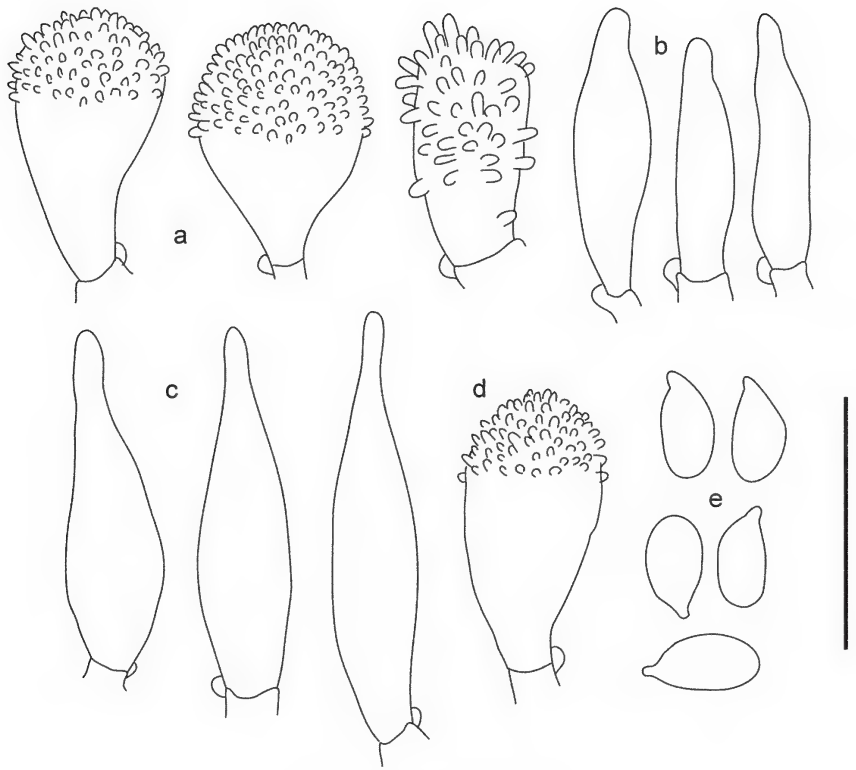

Fig. 4. "*Marasmius junipericola*".
a. Pileipellis cells, b. pileocystidia, c. cheilocystidia (type 2),
d. cheilocystidia (type 1), e. basidiospores. Scale bar = 20 µm.

Pleurocystidia rare, similar to cheilocystidia of type 2. Trama and context hyphae ± cylindrical, branched, thin- to slightly thick-walled, smooth or minutely incrusted, up to 8.0 µm wide. Pileipellis a hymeniderm composed of 16–28 × 9.0–16 µm, (broadly) clavate, vesiculose cells of the Rotalis-type, entirely thin-walled or with slightly thick-walled apex; mixed with scattered cells with larger diverticula or diverticulate hyphae. Pileocystidia 21–25 × 5.0–6.5 µm, ± fusoid, rostrate, thin-walled. Stipitipellis a cutis of cylindrical, parallel, distinctly thick-walled (walls up to 2.0 µm thick), mostly smooth, less frequently with scattered diverticula. Caulocystidia 20–40 × 3.0–6.0 µm, adpressed to erect, cylindrical, narrowly clavate, subfusoid, mostly slightly thick-walled. Clamp connections present in all tissues.

Chemical reactions — No part of basidiocarps amyloid or dextrinoid.

Ecology — On fallen needles of *Juniperus chinensis* var. *kaizuka*.

Specimen examined — **REPUBLIC OF KOREA**, Sobaeksan National Park, Sobaeksan Youth Hostel area, 16 July 2007 leg. J.G. Han (Antonín 07.59, BRNM 714677).

Remarks — "*Marasmius junipericola*" is similar to *M. crescentiae* but differs by the presence of well-developed caulocystidia. Although it represents a distinct taxon, the collection was too small (two minute basidiocarps) for a valid description of a new species. For similar taxa, see the discussion on *M. crescentiae*.

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We gratefully acknowledge Jan Holec (National Museum, Prague, Czech Republic) and Michal Tomšovský (Mendel University, Brno, Czech Republic) for critically reviewing this manuscript. The collecting trip to the Republic of Korea and the studies of the collected material by the first author were supported by the Czech Science Foundation (No. 206/07/J003). The other authors were supported by the Korea Research Foundation Grant funded by the Korean Government (KRF-2006-F00001), and research grant by Korea Forest Institute (FP 0801-2010-01).

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A new hairy species of *Leptogium* (*Collemataceae*) from China

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Abstract — This paper describes *Leptogium weii* sp. nov., a new hairy species from Sichuan, southwestern China. *Leptogium weii* has dense white tomentum on apothecial stalks and thalline margins. It closely resembles *L. burnetiae* in external morphology but differs in having globose isidia on the upper surface and dense tomentum on the apothecial stalk and thalline margin. A key is given to hairy species with tomentose apothecia.

Key words — lichen, *Ascomycota*, *Peltigerales*, corticolous

Introduction

Thirty-two *Leptogium* species have previously been reported in China (Wei 1991, Aptroot 2002, Wang et al. 2010). Here we describe a characteristically hairy new species, *Leptogium weii*.

Material & methods

The type specimen was collected from Sichuan Province in southwestern China, and deposited in Herbarium Mycologicum Academiae Sinicae-Lichenes (HMAS-L). A dissecting microscope (Motic SMZ-140) and a light microscope (Motic B2) were used for the morphological and anatomical studies.

Taxonomy

Leptogium weii H.J. Liu & S. Guan, **sp. nov.** Figure 1

MycoBank MB 519891

Differt a Leptogio burnetiae *isidiis globosis, et marginibus apotheciorum dense hirsutissimis; asci interdum quadrospori.*

Type: China. Sichuan Province, Mt. Gonggashan, Zimeishan (29°37′N 101°53′E), alt. 4000 m, on branch of *Rhododendron* sp. (*Ericaceae*), 29/VII/1982, Xian-Ye Wang, Xie Xiao & Bin Li 9082 (**Holotype**, HMAS-L 031713).

Etymology: The species is named in honour of Professor Jiang-Chun Wei.

Thallus foliose, 6–8 cm in diam. Lobes broadly rounded, 10–20 mm wide, apices entire, undulate; upper surface blue gray, usually shiny, glabrous, without wrinkles, isidiate; isidia globular, laminal to marginal, dense, darker than upper surface; lower surface pale to medium gray, smooth, with a dense covering of long-celled, white hairs, to 0.2 mm long, ± naked near margin. Apothecia ± dense, laminal to submarginal, 1.0–2.5 mm in diam., stipitate; disc dark red brown, concave and finally plane, glabrous, dull; stalk always with dense, suberect to erect, white tomentum; thalline margin smooth, paler than upper surface, non-isidiate, with similar tomentum on stalks, sometimes glabrous when old.

Thallus 90–110 μm thick when moist. Upper cortex paraplectenchymatous, consisting of a single layer of globular, isodiametrical cells, 4–6 μm thick; lower cortex paraplectenchymatous, commonly consisting of a single layer of ellipsoidal thin-walled cells, 8–10 μm thick, or 40–50 μm thick when below apothecia and consisting of 3–4 layers of cells; medulla 80–100 μm thick, composed of loosely interwoven hyphae running in various directions; photobiont *Nostoc* in chains, ± globose, 5–7 μm in diam., ± concentrated near both cortices.

Apothecia 300–400 μm thick (when moist); thalline exciple 100–180 μm wide; proper exciple not seen; epihymenium brown, 10–15 μm high; hypothecium yellowish, 40–50 μm high. Hymenium in its central part 160–210 μm high; paraphyses simple, 1–1.5 μm wide, at apices often thickened (2–3 μm); asci clavate, 125–150 × 20–30 μm, commonly 8-spored, sometimes 4-spored. Ascospores fusiform to ellipsoidal, apices obtuse or sometimes acute, 25–35 × 12–17 μm, transversely septate to submuriform, with 3–5 transverse and 0–1 longitudinal septa, colourless. Conidiomata not observed.

Habitat and distribution: Corticolous on branches of *Rhododendron* sp., in the alpine region of southwestern China.

Remarks – *Leptogium weii* is characterized by an upper surface with globular isidia and without wrinkles, long hairs on the underside, white tomentum on apothecial stalks and thalline margins, and (4–)8-spored ascus. It closely resembles *L. burnetiae* C.W. Dodge in external morphology. But the latter always has coralloid isidia, 8-spored ascus, and the apothecium is often isidiate on thalline margin (Jørgensen & Nash 2004, Aragón et al. 2004, 2005, Wang et

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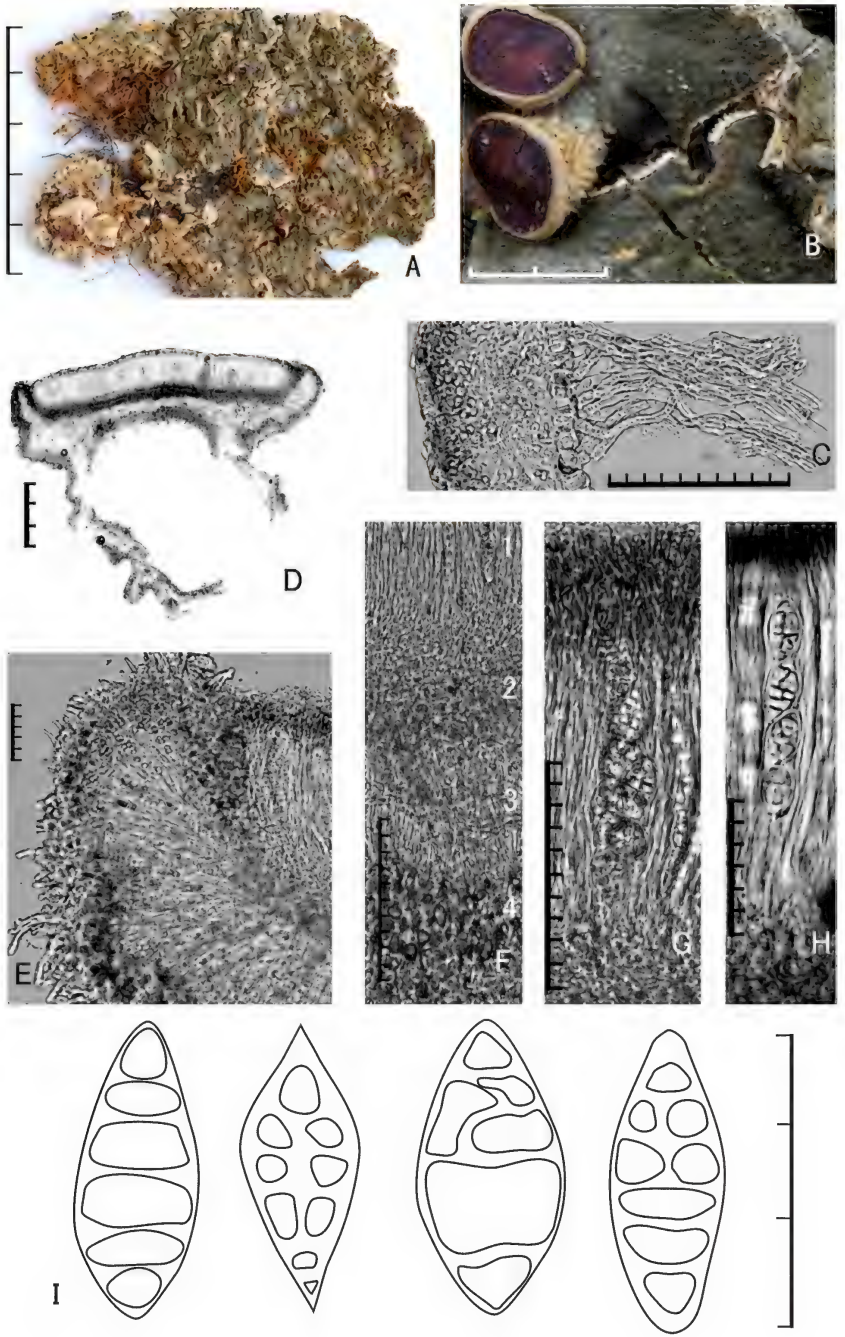
Fig. 1. *Leptogium weii* (holotype). A. Thallus with apothecia. B. Apothecia with tomentose stalk (note the globular isidia on upper surface). C. Thallus cross-section, showing corticated surfaces and long hairs on lower surface. D. Apothecium cross-section. E. Thalline margin (note the dense tomentum on the surface). F. Vertical section of the central part of apothecia (1 stands for hymenium, 2: subhymenium, 3: medulla, and 4: corticated lower surface). G. An ascus with 8 spores. H. An ascus with 4 spores. I. Spores. Scale bars: A = 1 cm; B = 1 mm; C, E–I = 10 μm; D = 100 μm.


A
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al. 2010). *Leptogium trichophoroides* P.M. Jørg. & A.K. Wallace and *L. askotense* D.D. Awasthi also have tomentose stalks and thalline exciples, and long hairs on the underside as observed in *L. weii*. But *L. trichophoroides* has wrinkles on a browned upper surface without isidia and smaller spores (18–22 × 9–12 μm) (Jørgensen 1997). *Leptogium askotense* differs from the new species in lacking isidia, having wrinkles on the upper surface and longer spores (30–50 × 10–17 μm) (Jørgensen 1997). *Leptogium sphaerosporum* P.M. Jørg. & Olley is differentiated by phyllidia (without tomentum on apothecial stalk and isidia) on the upper surface and sphaeroid spores (Jørgensen & Olley 2010), while *L. saturninum* is distinguished by a darker upper surface, sessile apothecia with a glabrous thalline exciple and euparaplectenchymatous proper exciple (Sierk, 1964, Jørgensen & Nash 2004).

The hairy species in *Leptogium* have been summarized by Jørgensen (1997). Here we present a key to 12 hairy *Leptogium* species with tomentum on apothecial stalks or thalline margins derived from keys by Jørgensen (1997), Jørgensen & Kashiwadani (2008), and Jørgensen & Olley (2010).

Key to hairy *Leptogium* species with tomentose apothecial stalks or thalline margins

1 Hairs on both sides of thallus ..... *L. australe*
Hairs only on lower surface of thallus 2

2 Upper surface distinctly wrinkled 3
Upper surface smooth or slightly rough, not wrinkled 9

3 Thallus isidiate ..... *L. papillosum*
Thallus not isidiate 4

4 Proper exciple distinct, euparaplectenchymatous 5
Proper exciple absent ..... *L. acutisporum*

5 Thallus normally bluish; apothecia sessile, margin wrinkled ..... *L. arisanense*
Thallus normally dark brownish or greenish 6

6 Apothecia with stiff marginal hairs 7
Apothecia without stiff marginal hairs, sometimes softly hairy on the stalk ..... *L. askotense*

7 Apothecia stalked, with white hairs when young ..... *L. trichophoroides*
Apothecia sessile or short stalked, usually with blackish hairs 8

8 Lobes strap-shaped ..... *L. loriforme*
Lobes short orbicular ..... *L. trichophorum*

9 Thallus isidiate 10
Thallus not isidiate 11

10 Isidia cylindrical coralloid; apothecia rare, often isidiate on margin *L. burnetiae*
Isidia usually granular; apothecia abundant, not isidiate on margin ..... *L. weii*

11 Apothecia prominently stalked ..... *L. pedicellatum*
Apothecia sessile or short stalked ..... *L. menziesii*

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Abrachium, a new genus in the *Clathraceae*, and *Itajahya* reassessed

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Abstract — Molecular and morphological analyses have elucidated phylogenetic relationships of two remarkable species in the *Phallales*: *Aseroe floriformis* and *Phallus roseus*. Genes from ATPase subunit 6 (atp6), the nuclear large subunit ribosomal DNA (nuc-LSU), and the second largest RNA polymerase II subunit (RPB2) underwent Bayesian and parsimony molecular analyses. Molecular datasets, combined with morphological characters, support a new genus (*Abrachium* for *Aseroe floriformis*), reassessment of *Itajahya,* and emendation of *Clathraceae.*

Key words — *Phallomycetidae*, gasteroid fungi, neotropics, phylogeny, evolution

Introduction

Fischer (1898-99) established the order *Phallales*, initially composed of two families, *Clathraceae* and *Phallaceae*, to separate representatives with branched and unbranched basidiomata. *Clathraceae,* established by Chevallier (1826), encompasses the "lattice stinkhorns", fungi that exhibit a peculiar basidioma featuring tubular or mucilaginous arms either interconnected or intertwined in a network or comprising a receptacle with vertical arms either connected at the top or arched in the shape of a star (Miller & Miller 1988). Pegler & Gomez (1994) included twelve genera within the family (*Blumenavia, Linderiella, Pseudocolus, Anthurus, Aseroe, Lysurus, Clathrus, Ileodictyon, Simblum, Colus, Neolysurus* and *Kalchbrennera*).

Corda (1842) proposed *Phallaceae* for species with fruiting bodies composed of a single, hollow receptacle with a foul-smelling gleba covering the apex. Morphological characters widely used to separate genera include

the presence of a campanula (head) at the apex of the receptacle in *Phallus*, indusium in *Dictyophora*, and calyptra in *Itajahya*. Some genera lack a distinct head and form the mucilaginous gleba directly on the apical receptacle (e.g., *Mutinus*) or at a constriction near the apex (e.g., *Staheliomyces*). Several attempts were made to establish a synopsis of the family (Lloyd 1909, Fischer 1933), and Cunningham (1944) included seven genera: *Dictyophora, Itajahya, Phallus, Mutinus, Staheliomyces, Aporophallus,* and *Floccomutinus*. However, Kreisel (1996) viewed *Dictyophora, Aporophallus, Itajahya, Echinophallus,* and *Endophallus* as subgenera of *Phallus* sensu lato, using the shape and surface of the head, pigmentation of the receptacle, and morphology of the volva and rhizomorphs as primary characteristics. He did not deem the presence of a pore at the receptacle apex or the morphology of the head, indusium, and calyptra as sufficient for segregation at the generic level (Kreisel 1996).

Taxonomic studies of Neotropical phalloid species have revealed new and intriguing species (Baseia et al. 2003, Baseia & Calonge 2005, Fazolino et al. 2010). The discovery of new species is essential to understanding the evolutionary path of *Phallales* clades, since according to known estimates (Hawksworth 2001, Mueller et al. 2007, Blackwell 2011), most tropical species remain unknown. Through morphological data and molecular tools the classification of macrofungi should change in coming years.

The present study aims to evaluate the actual taxonomic position of *Aseroe floriformis* and *Phallus roseus*, two species with distinct morphological features that have not yet been analyzed by molecular studies.

Materials & methods

Basidioma sampling

Basidiomata were sampled at the Parque Estadual Dunas do Natal (06°22′10″ S and 35°00′28″ W) and Estação Ecológica do Seridó (06°35′02″ S and 37°17′02″ W), Rio Grande do Norte state, Brazil. Specimens of *Aseroe floriformis* and *Phallus roseus* were photographed and collected during the rainy seasons in 2008 and 2009. Materials studied were deposited in the fungal collection of the UFRN Herbarium.

Macroscopic characters were described from fresh specimens. Basidiomata colors were based on Kornerup & Wanscher (1978). Microscopic characteristics were determined from temporary mounts, hand-sectioned with a razor blade on the vertical axis of the basidiomata and mounted in 3% KOH and 1% cotton-blue lactophenol, respectively. All measurements were made under oil-immersion microscope objective at 1000× magnification. Spore measurements included the largest and smallest spores and at least 20 additional randomly selected spores from each specimen.

Molecular techniques

Fresh basidiomata of *Aseroe floriformis* (UFRN 1481) and *Phallus roseus* (UFRN 535) were used for CTAB DNA extraction. The CTAB buffer (1.4M NaCl, 100mM Tris-HCl pH 8.20mM EDTA and 2% CTAB) was added to 0.8g of fresh tissue pulverized

with liquid nitrogen and incubated for 30 min. at 65°C. After incubation, a chloroform-isoamyl (24:1) extraction was performed, followed by isopropanol precipitation. Total DNA amount was quantified on a NanoDrop-2000 spectrophotometer (NanoDrop Technologies Inc. Wilmington, DE 1980, United States).

DNA sequences were obtained for three different regions: nuc-LSU-rDNA (LROR/LR5), ATPase subunit 6 (ATP61/ATP62) and the second largest subunit of RNA polymerase RPB2 (bRPB2-6F/bRPB2-7R), with previously designed primers (Vilgalys 1990; Liu et al. 1999; Kretzer & Bruns 1999).

All PCR reactions were carried out in a final volume of 50µl, with PCR buffer 10X, 4 mM of $MgCl_2$, 25 ng of DNA, 0.3 mM of dNTP Mix, 12.5 pmol of each primer and 0.3µl of Invitrogen® DNA Taq Polymerase (5U/µl). The initial cycling parameter for atp6 reaction condition consisted of 2 min at 95°C for initial denaturation, 5 cycles of 35 s at 94°C, 55 s at 37°C and 1min at 72°C. This was followed by 30 cycles of 35 s at 94°C, 55 s at 45°C, 1 min at 72°C, and final extension of 5 min at 72°C with final hold of 4°C. The nuc-LSU-rDNA amplification condition underwent initial denaturation for 2 min at 95°C, followed by 39 cycles for 1 min at 94°C, 30 s annealing at 45°C, 1 min extension at 72°C, and a final extension for 5min at 72°C. RPB2 amplifications followed conditions described by Liu et al. (1999).

Amplicons were verified by ethidium bromide-stained 0.4% agarose gel electrophoresis. Total amount of amplified DNA was quantified by a NanoDrop-2000 spectrophotometer. Amplifications with low DNA content were re-amplified by increasing PCR cycles.

PCR products were purified with Wizard® SV Gel and PCR Clean-Up System (Promega). The sequencing reaction was performed with a DNA sequencing kit (BigDye® Terminator v3.1 Cycle Sequencing Kit) using the same primers employed to amplify each fragment, and sequencing was conducted an ABI 3730 DNA Analyzer (Applied Biosystems). Both were carried out at the Center for Human Genome Studies (University of São Paulo, CEGH-USP) and later assembled using DNA Baser v3.0.12 beta.

Phylogenetic analysis

Phylogenetic analyses were performed for three combined DNA datasets under Bayesian and parsimony criteria. Newly generated sequences for *Aseroe floriformis* and *Phallus roseus* ATP6, nuc-LSU-rDNA and RPB2 and those retrieved from GenBank (National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov – Table 1) were separately aligned using ClustalX2 (Larkin et al. 2007), with default settings (alignment files are available at http://purl.org/phylo/treebase/phylows/study/TB2:S11546). Manual adjustments were made in BioEdit v. 7.0.9.0 (Hall 1999). The three alignments were then concatenated with Phyutility (Smith & Dunn 2008). Maximum parsimony analyses were conducted with combined dataset file, in PAUP* (Phylogenetic Analysis Using Parsimony) version 4b10 (Swofford, 2002). Heuristic search was used with tree bisection-reconnection (TBR) and MULTrees. Confidence levels were established through 1000 bootstrap replicates. Bayesian analysis was carried out with combined dataset in MrBayes v.3.1.2 (Huelsenbeck & Ronquist 2001). Two different runs with four incrementally heated simultaneous Monte Carlo Markov chains were conducted over 2 million generations, applying respective substitution models determined for

each partition in MrModeltest v.2.2 (http://www.abc.se/~nylander/). Trees were sampled every 100 generations to produce 20,000 trees. In order to estimate posterior probabilities, 25% of the trees were discarded as a burn-in stage, observing when average standard deviation of split frequency (ASDSF) values dropped below 0.01.

Table 1. GenBank accession numbers used for phylogenetic analysis.

Species	GenBank Accession Number		
	nucLSU	ATP6	RPB2
Anthurus archeri	DQ218624	DQ218913	DQ219081
Abrachium floriforme	JF968440	JF968438	JF968442
Aseroe rubra	DQ218625	DQ218914	DQ219082
Clathrus chrysomycelinus	DQ218626	DQ218915	DQ219083
Claustula fischeri K.M. Curtis	—	—	DQ219086
Dictyophora duplicata (Bosc) E. Fisch.	DQ218481	DQ218765	DQ219087
D. indusiata (Vent.) Desv.	DQ218627	DQ218917	DQ219088
D. multicolor Berk. & Broome	DQ218628	DQ218918	DQ219089
Gelopellis sp.	DQ218630	DQ218919	DQ219090
Gelopellis sp.	DQ218631	DQ218920	DQ219091
Ileodictyon cibarium	DQ218633	DQ218922	DQ219093
I. gracile	DQ218636	DQ218925	DQ219096
Itajahya rosea	JF968441	JF968439	—
Kobayasia nipponica (Kobayasi) S. Imai & A. Kawam.	DQ218638	DQ218926	DQ219098
Laternea triscapa	DQ218640	DQ218928	DQ219099
Lysurus borealis (Burt) Henn.	DQ218641	DQ218929	DQ219100
L. mokusin (L.) Fr.	DQ218507	DQ218791	DQ219101
Mutinus elegans (Mont.) E. Fisch.	AY574643	AY574785	DQ219102
**Phallobata alba* G. Cunn.	DQ218642	DQ218930	DQ219103
**Phallus costatus* (Penz.) Lloyd	DQ218513	DQ218797	DQ219104
P. hadriani Vent.	DQ218514	DQ218798	DQ219044
P. ravenelii Berk. & M.A. Curtis	DQ218515	DQ218799	DQ219105
Protubera borealis S. Imai	DQ218516	DQ218800	DQ219106
P. canescens	DQ218645	DQ218932	DQ219108
P. jamaicensis (Murrill) Zeller	DQ218647	DQ218933	DQ219110
P. maracuja Möller	DQ218518	DQ218802	DQ219111
P. parvispora Castellano & Beever	DQ218648	DQ218934	DQ219112
P. sabulonensis Malloch	DQ218649	DQ218935	DQ219113
Simblum sphaerocephalum Schltdl.	DQ218521	DQ218806	DQ219115
**Trappea darkeri* (Zeller) Castellano	DQ218651	DQ218938	DQ219116
Pseudocolus fusiformis	AF213128	—	—

* Species used as outgroup

Results

Phylogenetics analysis

Bayesian and maximum parsimony analyses produced no significant differences in tree topology, indicating data consistency to resolve the phylogeny of the group. The phylogram (Fig. 3) illustrates six well-formed clades corresponding to the families of *Phallales* with high support values (PP = 1, BT = 90–100). Intergeneric relationships in each family are also shown. It was found that *Aseroe floriformis* does not cluster with *Aseroe rubra*, and occupies a basal position in the *Clathraceae*, with PP = 1 and BT = 99. In *Phallaceae*, *Phallus roseus* occupies a position between *Mutinus elegans* and other *Phallus* species, including indusiate representatives (*Dictyophora*), which cluster into a single clade displaying high levels of reliability (PP = 1, BT = 94).

Taxonomic revision

Based on the morphological and molecular data set, we propose a new genus to accommodate *Aseroe floriformis*. The most important morphological feature of the new genus —the lack of arms— requires an emendation in *Clathraceae*. Phylogenetic analyses also support the status change of the genus *Itajahya* in *Phallaceae*.

Clathraceae Chevall. **emend.** Baseia

Emended description: Basidiomata sessile to shortly stalked, upright receptacle, spreading, sometimes arched and united arms or a latticed receptaculum composed of interconnected tubular or gelatinous arms expanding from a flaccid button, which acts as a volva, or armless sunflower-shaped receptacle. Gleba located on the central disc of receptacle or inside and next to the clathrate receptaculum. Peridium three-layered. Basidiospores narrowly elliptical, covered with mucilaginous layer.

Type genus: *Clathrus* P. Micheli ex L.

Other genera: *Abrachium* Baseia & T.S. Cabral, *Aseroe* Labill., *Blumenavia* Möller, *Pseudocolus* Lloyd, *Lysurus* Fr., *Ileodictyon* Tul. & C. Tul.

The genus *Aseroe* was proposed by Labillardière (1800), with *Aseroe rubra* Labill. as the type (and only) species. Fischer (1890) subsequently described *A. arachnoidea*. Yoshimi & Hongo (1989) added *A. coccinea*, a name recently validated by Kasuya (2007). Baseia & Calonge (2005) discovered a new species, *Aseroe floriformis*, in northeastern Brazil in sand dunes. Although Index Fungorum lists 27 names in *Aseroe*, the latest edition of the Dictionary of Fungi (Kirk et al. 2008) lists only two: *A. floriformis* and *A. rubra*. The genus has a pantropical distribution, although *A. rubra* has been reported in Europe (Spooner 2005). *Aseroe* is characterized by a cylindrical receptacle and stipe

that emerge from a whitish volva. With the exception of *Aseroe floriformis*, all species have apical free arms internally covered by the gleba (Dring 1980).

Abrachium Baseia & T.S. Cabral, **gen. nov.**

MycoBank MB 561618

Ovo subglobosae, epigaeo, rhizomorphis basalis. Stipite cylindrico, spongioso. Receptaculum floriformis cum disco sine ramulis, centralis perforatae cum gleba gelatinosa marginata, foetida. Sporis cylindraceo-bacilliformis, laevis, hyalinae.

Type species: *Abrachium floriforme* (Baseia & Calonge) Baseia & T.S. Cabral

Etymology: *Abrachium*, due to the absence of arms.

Key characters: Egg subglobose, epigeous with several and basal mycelial cords, white. Stipe cylindrical, spongy. Receptacle with the same anatomy, sunflower-shaped, without arms, central disc covered by a gelatinous gleba. Spores cylindrical to bacilloid, smooth, hyaline.

Abrachium floriforme (Baseia & Calonge) Baseia & T.S. Cabral, **comb. nov.** Fig 1

MycoBank MB 561619

≡ *Aseroe floriformis* Baseia & Calonge, Mycotaxon 92: 170, 2005.

Specimens examined: **BRAZIL, Rio Grande do Norte**, Natal, Parque das Dunas, ad solum arenarius, 3-VII-2004, legit I.G. Baseia & P.P.T. Lacerda, UFRN-Fungi 1851 (holotype); 07-V-2009, legit I.G. Baseia UFRN 1481, GenBank JF968440, JF968438, JF968442.

Our phylogenetic analyses confirm high support values for *Phallales* in accordance with Hosaka et al. (2007). Morphological and molecular analyses supported the establishment of the genus *Abrachium*, named for its armless receptacle (Fig 1). This taxon was originally described in the Brazilian Atlantic rainforest (Baseia & Calonge 2005), but has since been recorded in northeastern Brazil (Trierveiler-Pereira & Baseia 2009, 2011, Bezerra et al. 2009).

The clathraceous clade comprises members with a variable morphology of the basidiomata, consisting of tubular or gelatinous arms either interconnected or free (Dring 1980). The armless receptacle of *Abrachium* justifies an emendation in *Clathraceae* and seems to be a primitive characteristic of the clade, as shown in the tree obtained by molecular inference (Fig. 3). Molecular data indicate that receptacle morphology is a phylogenetically informative characteristic in *Clathraceae*. In phylogram, *Abrachium* represents a basal clade, which is a sister group of a clade formed by other *Clathraceae* species. The latter have a bifurcation leading to two other subgroups. The first subgroup either has arms attached at the ends [*Pseudocolus fusiformis* (E. Fisch.) Lloyd], or free and arched like a star [*Aseroe rubra* and *Anthurus archeri* (Berk.) E. Fisch.]. The second subgroup displays a complex structure, forming a "cage" type configuration including *Clathrus chrysomycelinus* Möller, *Laternea triscapa* Turpin, *Ileodictyon cibarium* Tul. & C. Tul., *Ileodictyon gracile* Berk.,

FIGURES 1–2. Fresh specimens: 1. *Abrachium floriforme*. 2. *Itajahya rosea*. Bar = 10 mm.

and *Protubera canescens* G. W. Beaton & Malajczuk. Based on these data, we can infer that the evolution to receptacle bifurcation (forming arms, e.g., *Aseroe*) occurred at least once in the Neotropical region. Presumably, after the divergence of *A. floriforme*, a common ancestor has diverged to forms where the receptacle may bifurcate like arms in one group, and form a cage in the other (such as *Ileodictyon*).

Itajahya rosea (Delile) E. Fisch., Ber. Deutsch. Bot. Ges. 47: 294, 1929. FIG. 2

≡ *Phallus roseus* Delile, Flore d'Égypte: 300, 1813.

SPECIMEN EXAMINED: **BRAZIL, RIO GRANDE DO NORTE**, Serra Negra do Norte, Estação Ecológica do Seridó, ad solum arenarius, 23-V-2008, legit T.B.S. Ottoni, UFRN-Fungi 535; GenBank JF968441, JF968439.

Molecular data analysis of *Phallus roseus* points to the need for reassessing its current taxonomic status in the subgenus *Itajahya* proposed by Malençon (1984) and Kreisel (1996). *Itajahya* was established by Möller (1895) based on *I. galericulata* Möller, a taxon discovered in Santa Catarina state, Brazil. The main feature distinguishing *Itajahya* from other *Phallaceae* genera is the presence of a structure called a calyptra (FIG. 2) located at the apex of the gleba (Möller 1895, Malençon 1953, Ottoni et al. 2010). Molecular data demonstrated that *P. roseus* diverged after *Mutinus* and prior to other species of *Phallus* (FIG. 3). Since *I. rosea* does not cluster with any other *Phallus* species, we propose

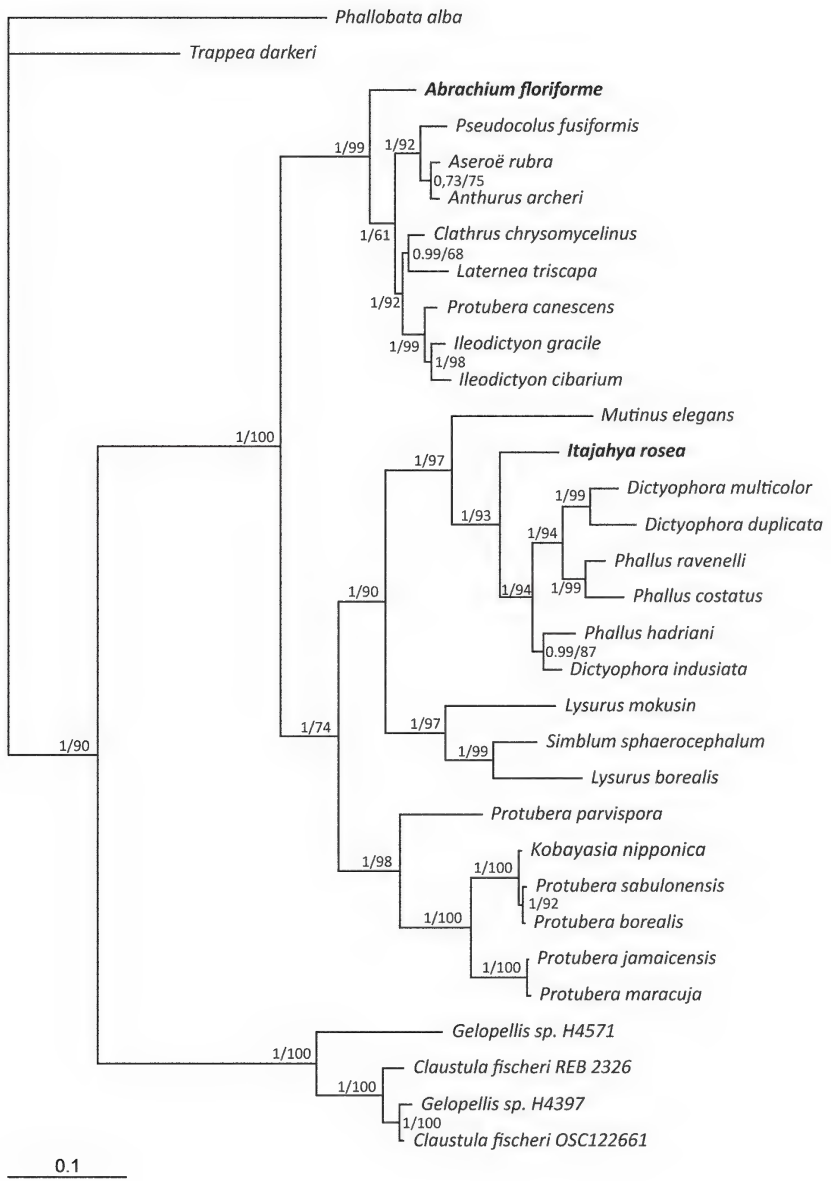

FIGURE 3. Phylogenetic tree of *Phallales* obtained through Bayesian analysis of atp6, nuc-LSU rRNA, and RPB2 sequences. Numbers preceding '/' = Bayesian posterior probability values (PP) and after '/' = maximum parsimony bootstrap values (BT).

raising *Itajahya* to generic status. Further collections and molecular studies of *I. galericulata* (= *Phallus galericulatus* (Möller) Kreisel) are also needed in order to ensure morphological characteristics (mainly the presence of calyptra) agree with molecular data recognizing the genus *Itajahya*.

Molecular data also imply that the indusium is not a phylogenetically informative character in *Phallaceae*. Malençon (1984) and Kreisel (1996) stated that species may be closely related with or without this structure, and that a species may or may not produce it or have a rudimentary form of it. The phylogram indicates that although *Dictyophora multicolor* and *D. duplicata* form a separate clade, *D. indusiata* clusters with *Phallus hadriani*, exhibiting prior divergence with the other mentioned clade. This suggests that indusiate species may have emerged independently during several phases of the group's evolution, thereby characterizing it as a polyphyletic assemblage. The phylogram also illustrates that *Mutinus* is a more primitive genus in *Phallaceae*, as observed by Cunningham (1944), based on data from simple sporophore morphology and the absence of complex structures such as campanulate apex, indusium and calyptra. It is important to note that further molecular studies are needed, especially with key-taxa that may lead to a change in current classification and give us a clearer picture of the systematics and evolution of phalloid fungi.

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Nomenclatural notes 13. An incorrect neotype designation and provision for a lectotype and an epitype for *Helvella fusca*

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Abstract — A neotype designation for *Helvella fusca* proposed in 1997 proves to have been erroneous because an illustration accompanying the original description was overlooked. Such illustrations normally have served as the lectotype when all known specimens of the author's taxon have been lost. The authors of that neotype designation should instead have designated the illustration as a lectotype and a specimen as an epitype, which is done in this paper. The so-called neotype specimen is now selected as the epitype specimen. A full description, illustrations and remarks about synonyms and a non-synonym are also provided.

Key words — International Code of Botanical Nomenclature

Helvella fusca was described by Gillet (1879: 9), but unfortunately his description did not specify any specimen or figure to represent that species, making typification more difficult. Dissing (1966b) wrote about this species: "I have not seen any authentic specimen of *H. fusca* Gillet, whereas the many Bresadola-collections of *H. fusca* in the herbarium in Stockholm (S) make it possible to get a precise concept of Bresadola's species." With respect to the type specimen of *H. fusca*, Dissing (1966b) wrote only, "not seen." Later, in their seminal paper on the northern and northwestern *Helvellaceae* of North America, Abbott & Currah (1997) made a minor but (we believe) critical error by designating a neotype specimen for *H. fusca,* even though that species does not occur in North America. They overlooked the excellent illustration in Gillet's paper (pl. 4, not cited in the species description but referenced mistakenly in one plate listing as pl. 14 and correctly in the other as pl. 4). They may have been unaware that such an illustration, if one upon which it can be shown that the description

Figs. 1–2. 1: Type of *Helvella fusca*, reproduced from Gillet (1879). 2: Part of *Helvella fusca* epitype (Bresadola's specimen 05 21 1898, S). Scale bar = 1 cm.

or diagnosis validating the name was based, is considered part of the original material (McNeill et al. 2006: Vienna Code Art. 9 Note 2) and becomes eligible as a lectotype (sometimes informally referred to an "iconotype") of the species name (Fig. 1). An interesting article by Ross (2002) brings into question whether an illustration accompanying an original description is always part of the original material and thus eligible as a lectotype when all specimens are lost or missing. At the XVIII International Botanical Congress in 2011 one proposal attempting to clarify such situations (Art. 9, Prop E (215); see McNeill & Turland 2011, Perry 2010) regrettably was rejected by a card vote (McNeill et al. 2011). We feel that this case should be corrected at a future Congress but now proceed under the assumption that Gillet's plate was drawn from living specimens now lost and that the illustration is part of the original material that can and should be designated as a lectotype.

A neotype may only be proposed when ALL original material is lost [Vienna Code]:

> Art. 9.6. A neotype is a specimen or illustration selected to serve as nomenclatural type if no original material is extant, or as long as it is missing."

Also pertinent is Art. 9.7:

> An epitype is a specimen or illustration selected to serve as an interpretative type when the holotype, lectotype, or previously designated neotype, or all original material associated with a validly published name, is demonstrably ambiguous and cannot be critically identified for purposes of the precise application of the name of a taxon.

The senior author pointed out this discrepancy to the junior author, and we have chosen instead to propose as epitype the same specimen designated as neotype by Abbott & Currah (1997) from the Bresadola collection in the Naturhistorische Rijksmuseum in Stockholm, Norway —Al Deserto, [Italy], collected by Bresadola, 05 21 1898 (FIG. 2). This specimen is also illustrated by Dissing (1966b). Oddly, Abbott & Currah failed to cite (or were unaware of) Bresadola's careful description, which clearly cited Gillet's figure and provided a superb plate of his own collection (Bresadola 1900: 100, tab. CCXII) (FIG. 3). That plate and description was reissued (Bresadola 1933: 1174, pl. 1174) with the headers and footers of the plate in different typeface and numbering and with a number of emendations of the text —notably eliminating Bresadola's 1900 acknowledgement that Boudier had informed him that specimens Bresadola had sent to him were identical with Gillet's species. The 1933 plate renders the figures in paler colors throughout.

Since Gillet's plate does not provide enough information for modern identification, an epitype is in order. The CODE requires that if an illustration is the only surviving element of the original material, that illustration should be designated as a lectotype, which we do in this paper. The confused information that already exists on the morphology of this species in the literature can be resolved with an epitype specimen. Gillet (1879) described *H. fusca* with spherical to subspherical ascospores, and he did not mention any other useful microscopic character. Bresadola (1900) described ascospores as ellipsoidal, 18–20 × 12–13 µm (but he did not indicate whether those measurements correspond to smooth or to verrucose ascospores), and a glabrous, sterile outer surface of the apothecium. Dissing (1966b) mentioned ascospores 17–18.4–21 × 11–12–13 µm, with pustules when young, and the outside of the apothecium naked or very delicately pubescent. The senior author studied Bresadola's specimen 05 21 1898 (now the epitype) and observed that smooth (young) ascospores were larger, while verrucose (mature) were smaller (FIG. 5a) (see species description), with the hyphal fascicles of the apothecium absent or ≤ 50 µm in length (FIG. 5b), corresponding to a glabrous to subpubescent surface.

Inasmuch as the incorrect neotypification appeared in Mycotaxon (Abbott & Currah 1997: 61), we have chosen that same journal in which to rectify their action and to propose both the new lectotype and new epitype for the name.

The corrected typifications and full description

Helvella fusca Gillet, Champignons de France, Discom.: 9, pl. 4, 1879. Fig. 5

Type: Pl. 4, in Gillet, Champignons de France, Discom., 1879 (**Lectotype, designated here**); Bresadola 05.21.1898, Al Deserto, Italy (S) (**Epitype. designated here**).

= *Helvella fusca* var. *bresadolae* Boud., Icon. Mycol. (Paris), Tome 2: pl. 230, 1910; Tome 4: 121, 1911.

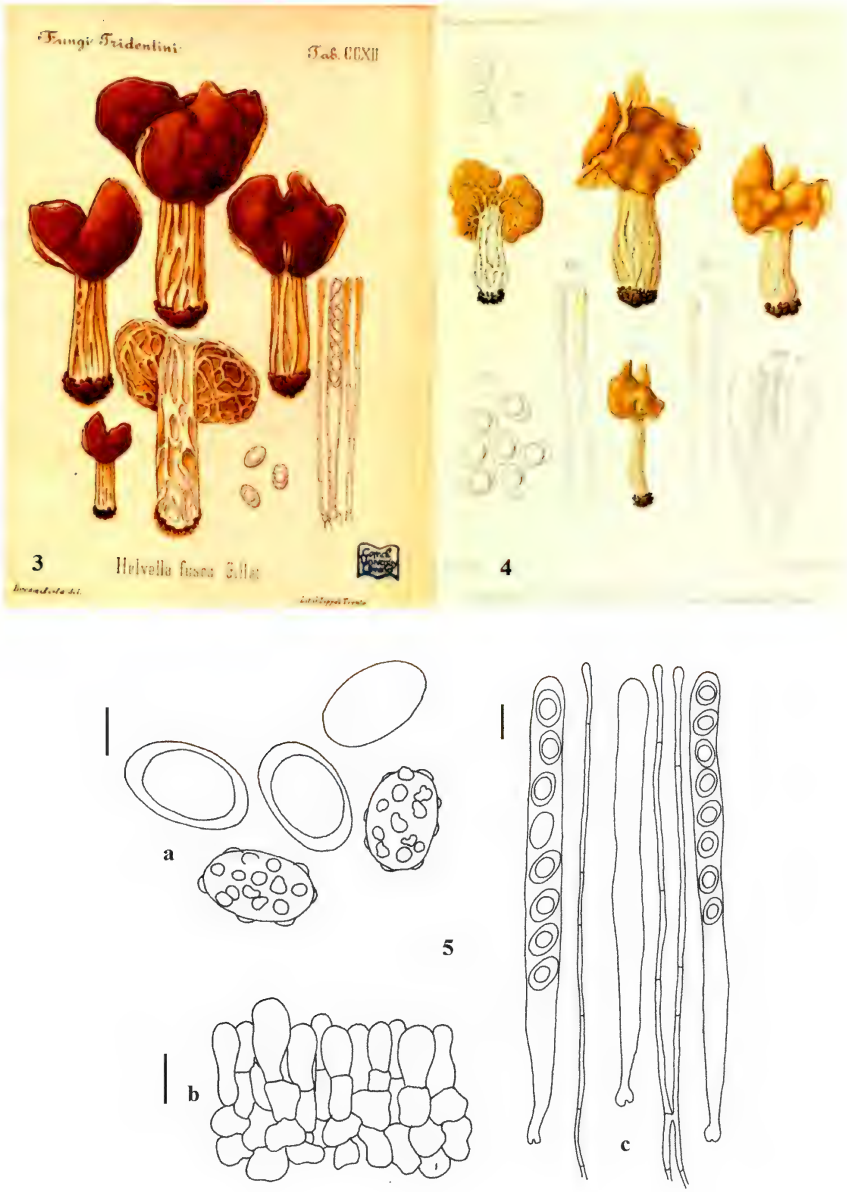
= *Helvella fusca* var. *gyromitroides* Chenant., in Pelé & Chenantais, Bull. Soc. Sci. Nat. Ouest, Sér. 4, 1: 75, 1921.

≠ *Helvella sulcata* α *fusca* Afzel., K. Vetensk.-Acad. Handl. 4: 305, Tab. X, fig. 1, 1783.

Apothecium 6–25 mm diam., 4–25 mm high, irregularly lobed or irregularly saddle shaped, margin fused to the stipe or rarely free, hymenium pale brown, tawny, to dark brown when dry, outside glabrous to subpubescent, whitish, yellowish, to pale brown, ribs branched or unbranched, extending near to the marginal zone of the excipular surface. Stipe 8–45 mm high, 3–15 mm broad, equal or slightly wider at the base, whitish or pale brown, glabrous to subpubescent, lacunose, internally chambered.

Asci 260–320 × 14–15.5 µm, pleurorhynchous. Ascospores young, smooth: (17–)18.5–21 × 11–13 µm; mature, verrucose: 15.5–18.5 × 11–12.5 µm. Paraphyses clavate, 5–8 µm wide at the apex, simple or branched, septate, thin-walled, yellowish brown, pigmented deposits in the cytoplasm. Medullary excipulum hyaline. Ectal excipulum yellowish brown, pigmented deposits usually in the cell wall and in a few cells in the cytoplasm. Inner layer of the stipe hyaline. Outer layer of the stipe yellowish brown, pigmented deposits usually in the cell wall and in few cells in the cytoplasm. Hyphal fascicles of the apothecium and stipe up to 50 µm long, light brown in mass, pigmented deposits in the cell walls. None of the pigmented deposits in any of the structures are visible in cotton blue mounts. All tissues are "rr–."

Remarks — *Helvella fusca* can be confused with *H. lacunosa* Afzel., because both have lobed apothecia, a lacunose stipe, glabrous to subpubescent excipular surface with the outer surface of the stipe similar, but *H. fusca* has a pale brown hymenium and grows associated with *Populus* spp., while *H. lacunosa* has a black or greyish hymenium and has a global distribution with no particular association with *Populus*. Boudier (1904) first introduced *H. fusca* var. *bresadolae* as a nomen nudum for a plate he planned to issue later. As pointed out by Korf (1986: 213, footnote; 1988: 213, footnote), Boudier (1907) soon after abandoned the variety, citing his plate as merely *H. fusca*. Boudier (1910), who eventually validly proposed *H. fusca* var. *bresadolae* (Fig. 4) based on apothecia



Figs. 3–5. 3: *Helvella fusca* reproduced from Bresadola (1900, from Boudier's book deposited in Cornell University Library). 4: Type of *H. fusca* var. *bresadolae* reproduced from Boudier (1910, Tome 2, pl. 230). 5: *Helvella fusca* (epitype), a: ascospores, b: ectal excipulum, c: paraphyses and asci. Scale bar: 5a = 8 µm; 5b, 5c = 20 µm.

that Bresadola sent him (but unfortunately not cited in the description), wrote "Hæc varietas typo simillima, differt tantummodo pediculo non albido, sed pileo concolore." Boudier (1911) later expanded the description. Dissing (1966a) considered this variety as invalid, because this specimen is possibly part of the Bresadola material that he studied and determined as "in all details identical." We also do not recognize the variety, because Gillet did not describe the stipe color only as white but as "dessous pâle, blanc sale ou lég[èremen]t. fuligineux." Furthermore, in *Helvella* stipe coloration varies too greatly within a species to be considered useful for recognizing varieties. For example the stipe of *H. lacunosa* can vary from whitish to grayish to black. Chenantais (Pelé & Chenantais 1921) proposed *H. fusca* var. *gyromitroides*, but the features that he used to recognize the variety (multilobed, fawn and free apothecial margin, and lacunose and yellowish stipe) are within the range of variation of the species. We do not consider *H. fusca* a synonym of *H. sulcata* α *fusca*, because Afzelius recognized *H. sulcata* with "sulcis regularibus," i.e., only longitudinal ribs on stipe (Afzelius 1783, Tab. X, fig.1; this figure is also in Dissing (1966b, fig. 27c), while *H. fusca* and *H. lacunosa* have longitudinal and transverse ribs (lacunose stipe) [i.e., in the words of Afzelius: "lacunis sulcisque irregularibus"]. DNA sequences support *H. sulcata* as a distinct species (unpublished data), contrary to the opinions by Dissing (1966b) and Abbott & Currah (1997), who considered it a synonym of *H. lacunosa*. Persoon (1801) described *H. sulcata* α *fusca* with "pileo nigro." *Helvella fusca* has more recently been reported from Europe, North America, and Asia (Dissing 1966b, Ceccaldi 2006, Kempton & Wells 1970; Kaushal 1991). Abbott & Currah (1997) doubted the existence of *H. fusca* in North America because their study indicated that such reports were probably *H. maculata* N.S. Weber. On the other hand, Kaushal (1991) recorded *H. fusca* from the Himalayas, but as his description mentioned hyphal fascicles of the apothecium measuring ≤ 105 µm long, it is unclear whether that report corresponds to *H. fusca*. Based on the above information, we can say that its distribution outside of Europe remains doubtful.

Acknowledgments

The authors thank John McNeill and Walter Gams, who have served as pre-submission reviewers. We also wish to acknowledge the efforts of the organizers of the July 2011 VII Congress of Latin American Mycology for providing an opportunity to meet each other and to begin a collaboration on studies of *Helvella* and other operculate discomycetes. The senior author thanks Laura Guzmán-Dávalos for her support and direction in his Doctoral studies and for revision of a preliminary version of this paper, and thanks Anders Tehler, Curator of the Herbarium S, for the loan of Bresadola's specimen. Virginia Ramírez Cruz (Universidad de Guadalajara, Mexico) is acknowledged for her valuable help inking the drawings. We both appreciate those responsible for the digital library of the Real Jardín Botánico de Madrid (http://bibdigital.rjb.csic.es/spa/index.php) as well

as the Internet Archive (http://www.archive.org/) for uploading invaluable literature for taxonomists on their websites.

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Herpothallon weii, a new lichen from China

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Abstract — A new species, *Herpothallon weii*, is described from China. This species is characterized by a byssoid whitish green thallus tightly attached to the substratum, pinkish pseudisidia, and the presence of psoromic acid and unknown substances.

Key words — biodiversity, *Arthoniaceae, Cryptothecia*, taxonomy

Introduction

Herpothallon (*Arthoniaceae, Arthoniales, Arthoniomycetes, Ascomycota*) was established by Tobler in 1937 and reinstated by Aptroot et al. (2009). The lichen genus is characterized by a crustose thallus without upper cortex, a byssoid prothallus, and a trentepohlioid photobiont. *Herpothallon* species often have pseudisidia or pseudisidioid structures, calcium oxalate crystals, pigments, and various lichen substances, but usually lack ascocarps. *Cryptothecia* species, which are morphologically similar to *Herpothallon* species, have an I+ blue medulla. Of *Herpothallon*, only six species (*H. biacidum, H. flavominutum, H. granulosum, H. isidiatum, H. philippinum,* and *H. rubroechinatum*) have an I+ blue medulla (Aptroot et al. 2009, Frisch et al. 2010a,b, Jagadeesh & Sinha 2009, Jagadeesh et al. 2009).

Herpothallon includes 35 species worldwide (Aptroot et al. 2009, Frisch et al. 2010a,b, Jagadeesh & Sinha 2009, Jagadeesh et al. 2009). In China, three *Herpothallon* species, *H. echinatum, H. granulare,* and *H. philippinum* have been reported (Aptroot et al. 2009). During our study of the lichen flora of Mt. Leigong in Guizhou province, China, a species of *Herpothallon* new to science was found.

Materials & methods

The specimens studied were collected from Guizhou, China, and are preserved in SDNU (Lichen Section of Botanical Herbarium, Shandong Normal University).

The morphological and anatomical characters of the specimens were examined using a stereo microscope (COIC XTL7045B2) and a polarizing microscope (OLYMPUS CX41). Lichen substances in the specimen cited were identified using the standardized thin layer chromatography techniques (TLC) with C system (Orange et al. 2010). Photos of the thalli were taken using a Olympus SZX12 with DP72.

Taxonomic description

Herpothallon weii Y.L. Cheng & H.Y. Wang, **sp. nov.** FIGS 1–2

MycoBank MB 563539

Differs from *Herpothallon albidum* by pinkish pseudisidia and lack of pigmentosin D.

TYPE: China. Guizhou province, Mt. Leigong, on bark, alt. 1000 m, 2 Nov. 2009, Q. Tian 20103366. (**Holotype** in SDNU).

ETYMOLOGY: In honour of the 80th birthday (6 Nov. 2011) of our dear teacher, Prof. Jiang-Chun Wei, who as the 'father of lichenology in China' has made a preeminent contribution to the development of lichenology and the education of Chinese talent in this field.

Thallus corticolous, crustose, tightly attached, ≤ 1.5 cm in diam., without upper cortex, distinctly byssoid, whitish green, with irregular calcium oxalate crystals. Prothallus distinct, ≤ 0.5 mm wide, white, byssoid, composed of radiating hyphae. Pseudisidia (also called pseudisidioid structure) numerous, pinkish, irregularly cushion-shaped, fluffy-felty with erect hyphae, ≤ 1 × 0.5 mm. Photobiont cells single or a few cells aggregated. Apothecia and pycnidia not seen.

CHEMISTRY — Thallus K+ yellow, C± yellow, KC+ yellow, P+ yellow, I–, UV–. Psoromic acid and unknown substances present.

DISTRIBUTION AND SUBSTRATE — *Herpothallon weii* is known only from the type locality; on bark.

ADDITIONAL SPECIMEN EXAMINED — **CHINA. GUIZHOU PROVINCE**, Mt. Leigong, on bark, alt. 1000 m, 2 Nov. 2009, Q. Tian, 20103174 (SDNU).

COMMENTS — Other *Herpothallon* species with pseudisidia and psoromic acid include *H. australasicum, H. echinatum, H. flavominutum, H. globosum*, *H. rubroechinatum,* and *H. albidum*. In contrast to *H. weii, H. australasicum* has a red thallus with concolorous compact pseudisidia; *H. echinatum* has soredioid fragments and concolourous pseudisidia and thallus; *H. flavominutum* has a greyish yellow thallus, granular pseudisidia, and lichexanthone, norlichexanthone, and psoromic acid; *H. globosum* has a red thallus, globose pseudisidia, and chiodectonic acid (red pigment); and *H. rubroechinatum* has an orange to red thallus, pseudisidia with red hairs, and common pycnidia. The more closely related *H. albidum* possesses very similar pseudisidia (also called pseudisidioid structures) but has white, rather than pinkish, pseudisidia and possesses pigmentosin D.

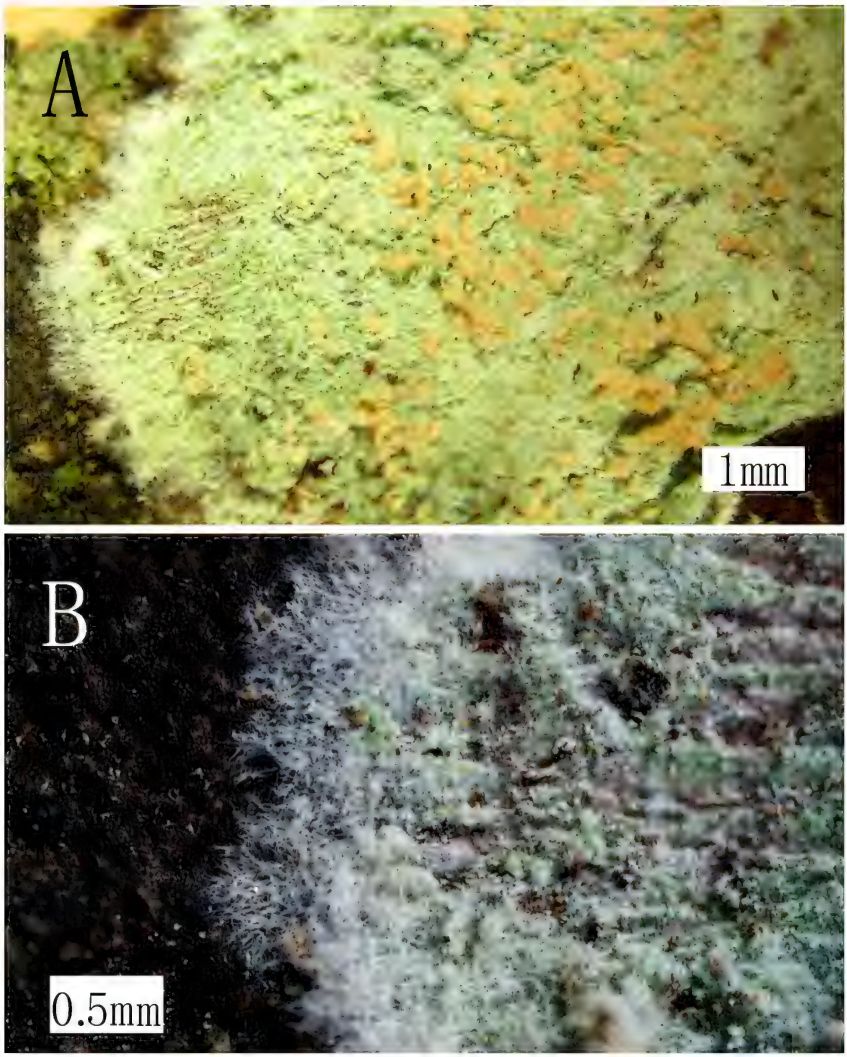

FIG. 1. *Herpothallon weii* (holotype). A. Thallus; B. Prothallus.

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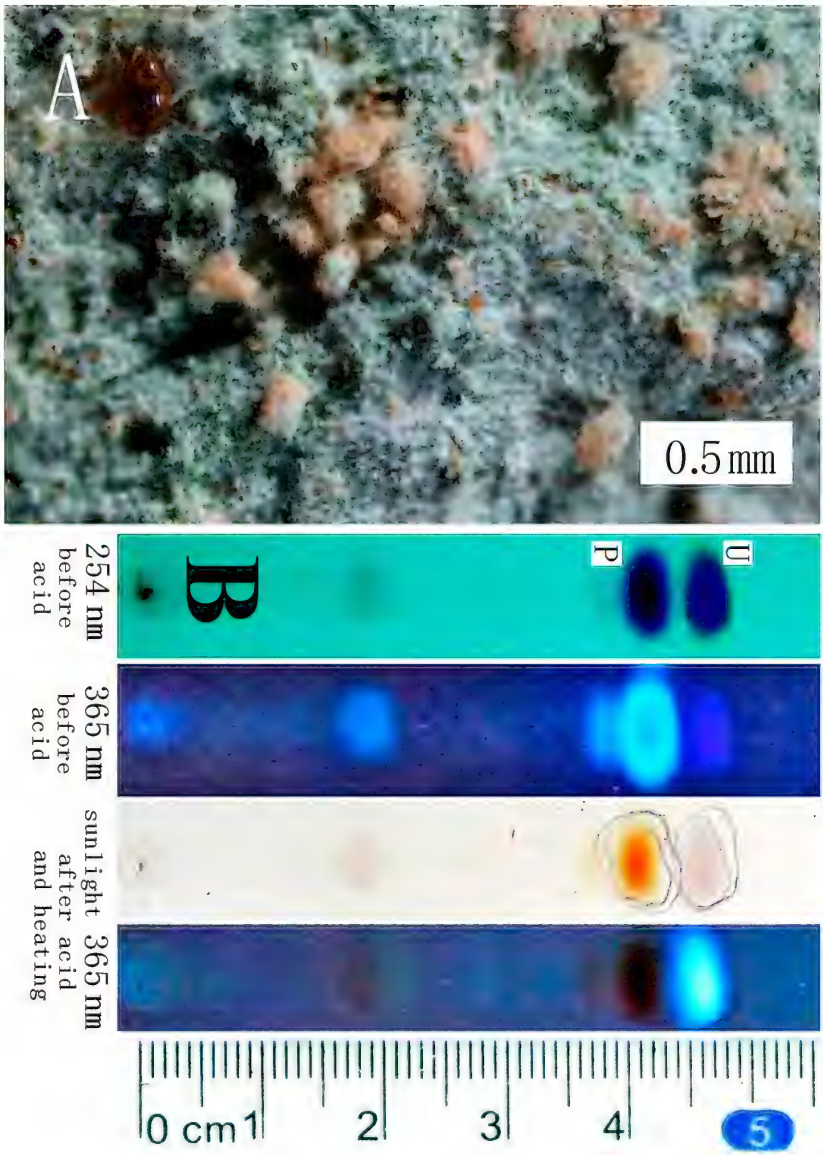

FIG. 2. *Herpothallon weii* (holotype).
A. Pseudisidia; B. TLC of *H. weii* (U: unknown substance; P: psoromic acid).

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MYCOTAXON

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Four new records of lecideoid lichens from China

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Abstract — Four lecideoid lichen species, *Carbonea vorticosa*, *Lecidea diducens*, *L. promiscens,* and *Lecidella bullata,* are reported for the first time from China.

Key words —Yunnan, Xizang, *Lecanoromycetes*, taxonomy

Introduction

As once one of the largest lichen genera, *Lecidea* s. lat. has had an extraordinarily broad circumscription, with Zahlbruckner (1926) accepting more than 1350 species. Subsequently, *Carbonea, Lecidella,* and many other obviously more natural units have been excluded based on the ascomal structure, especially the nature of the hamathecial tissues, ascus apical structures, and exciple (Hertel 1977, 1995; Smith et al. 2009). *Lecidea* s. str. has now become a medium-sized genus that lacks algae in the exciple and has a *Lecidea*-type ascus (Hertel 1995, Pérez-Ortega & Etayo 2008). *Carbonea* is characterized by a "carbonized" exciple and *Lecanora*-type ascus (Hertel 1983, Nash et al. 2004). *Lecidella* is characterized by *Lecanora*-type ascus and discrete paraphyses (Nash et al. 2004).

Worldwide, *Carbonea* includes 20 known species, *Lecidea* s. str. about 100 species, and *Lecidella* 79 species (Kirk et al. 2008). In China, these lecideoid lichens are still poorly known with only two *Carbonea,* 10 *Lecidella,* and 14 *Lecidea* s. str. species previously reported (Wei 1991; Abass & Wu 1998; Aptroot 2002, 2003; Obermayer 2004; Guo 2005; Zhang et al. 2010). During our study of lecideoid lichens from China, four species new to the country —*Carbonea vorticosa, Lecidea diducens, L. promiscens,* and *Lecidella bullata*— have been identified.

Materials & methods

The specimens studied were collected from Western China, and are preserved in SDNU (Lichen Section of Botanical Herbarium, Shandong Normal University), HKAS (Herbarium of Cryptogams, Kunming Institute of Botany Academia Sinica), or HMAS-L (Lichen Section, Herbarium of Mycology, Institute of Microbiology, Academia Sinica). Specimen morphology and anatomy were examined using a stereo microscope (COIC XTL7045B2) and a polarizing microscope (Olympus CX41). Lichen substances were identified using standardized thin layer chromatography techniques (TLC) with C and J system (Orange et al. 2010). Photos of the thalli were taken using a Olympus SZX12 with DP72.

The new records

Carbonea vorticosa (Flörke) Hertel, Mitt. Bot. Staatssamml. München 19: 442 (1983) FIG. 1A

Thallus crustose, indistinct, endolithic to areolate, thin; prothallus absent to not obvious; surface grey or white, rough. Apothecia sessile, up to 1.0 mm in diam.; disc black, plane to a little convex, epruinose; margin black, flexuous in older apothecia, long persistent; exciple blackish green to dark brown, hyphae radiating, 3.5–5 µm; epihymenium bluish green; hymenium hyaline with some green, 35–60 µm; hypothecium black to brown; asci clavate, 8-spored; ascospores hyaline, simple, oblong-ellipsoid, 10–13.5 × 3–5 µm.

SPOT TESTS — K–, C–, KC–, P–.

SECONDARY METABOLITES — none detected.

SPECIMENS EXAMINED — **CHINA. YUNNAN:** DEQIN COUNTY, Mt. Baimaxueshan, alt.4800 m, on rock, 13 Jul. 1981, L.S. Wang 12786 (HKAS); LIJIANG CITY, Mt. Laojun, alt. 4000m, on rock, 7 Nov. 2009, Y.L. Cheng 20100337-1, 20100289 (SDNU).

COMMENTS — *Carbonea vorticosa* is closely related to *C. capsulata*, which also produces small apothecia, a blue epithecium, and a brown hypothecium. However, *C. capsulata* has a more conspicuously blue hymenium and broader (5–10 µm diam.) excipular hyphae.

Reported from Antarctica, Asia, Australasia, Europe, and North and South America (Hertel 1991, Galloway 2007, Nash et al. 2004, Smith et al. 2009), *C. vorticosa* is new to China.

Lecidea diducens Nyl., Flora 48: 148 (1865) FIG. 1B

Thallus lacking or very indistinct, grey, not continuous; prothallus indistinct; medulla I+ deeply violet. Apothecia black, sessile, constricted at the base, up to 1.4 mm in diam.; disc black, flat to slightly convex, dull to shiny, epruinose; margin well developed, persistent; exciple with a blackish to dark brown rim and paler to hyaline interior, C+ red, K–; epihymenium blackish green; hymenium 45–50 µm, very pale green to hyaline; paraphyses simple, occasionally branched and anastomosing; subhymenium somewhat more intensely green than the

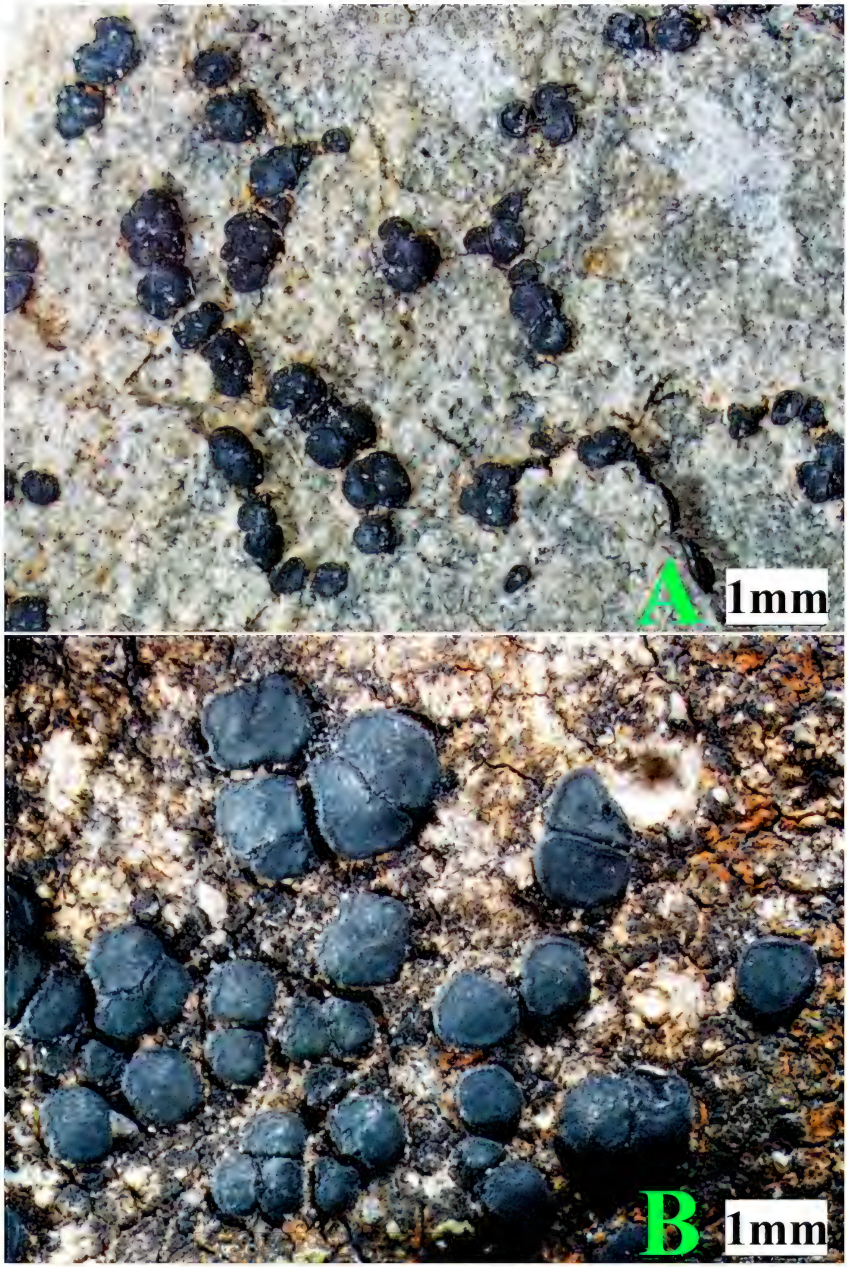

Fig. 1 A. *Carbonea vorticosa* (L.S. Wang 12786, HKAS);
B. *Lecidea diducens* (H.Y. Wang 20100297, SDNU).

hymenium, 10–30 µm thick; hypothecium pale brown to brown; asci clavate, 8-spored; ascospores simple, oblong-ellipsoid, 7.5–11 × 3.5–4 µm.

Spot tests — K–, C–, KC–, P–.

Secondary metabolites — 2'-*O*-methylanziaic acid.

Specimens examined — **China. Yunnan:** Lijiang county, Mt. Laojun, alt. 4000 m, on rock, 7 Nov. 2009, H.Y. Wang 20100332, 20100297 (SDNU).

Comments — *Lecidea diducens* is morphologically similar to *L. auriculata,* which lacks confluentic acid in the exciple and so is C–.

Probably cosmopolitan, *L. diducens* has been reported from Europe, North and South America, Asia, and Australasia (Hertel 1991, 2006, Hertel & Andreev 2003, Nash et al. 2004, Smith et al. 2009). New to China.

Lecidea promiscens Nyl., Flora 55: 358 (1872) Fig. 2A

Thallus grey, thin, not continuous; prothallus indistinct; medulla white, I+ deeply violet. Apothecia black, sessile, 0.8–1.8 mm in diam., disc black, flat to slightly convex, epruinose; margin black, well developed, persistent, often undulate; exciple with a thin blackish rim and hyaline to light brown interior; epihymenium greenish black to black; hymenium hyaline to pale green, 50–62 µm tall, I+ blue; paraphyses simple, occasionally branched, anastomosing; subhymenium hyaline to light brown; hypothecium brown to dark brown; asci clavate, 8-spored; ascospores hyaline, simple, oblong to oblong-ellipsoid, 7.5–12 × 4–4.5 µm.

Spot tests — K–, C–, KC–, P–.

Secondary metabolites — confluentic acid.

Specimen examined — **China. Yunnan:** Deqin county, Meilishi village, Suola Yakou, alt. 4700 m, on rock, 30 Aug. 2009, L.S. Wang 00-19783 (HKAS).

Comments — *Lecidea promiscens* is very similar to *L. auriculata* but has broader and larger spores. It also has a less prominent exciple (in *L. auriculata* the exciple extends quite far under the hypothecium), and a darker hypothecium.

Reported from Europe, North and South America, Asia, and Australasia (Thomson 1997, Hertel 1991, 2006, Nash et al. 2004), *L. promiscens* is new to China.

Lecidella bullata Körb., Parerga Lichenol.: 200 (1861) Fig. 2B

Thallus crustose, aerolate or bullate, moderately thick, grey-white to yellow-white, without soredia or isidia; prothallus absent. Apothecia sessile to semi-immersed, up to 1.5 mm in diam., disc black, flat to slightly convex, greyish pruinose to epruinose; margin black, at first prominent, later excluded; exciple greenish black outside and hyaline interior; epihymenium blue-green to black-green; hymenium hyaline, 40–55 µm tall, I+ blue; paraphyses simple; subhymenium and hypothecium hyaline, with crystals; asci *Lecanora*-type,

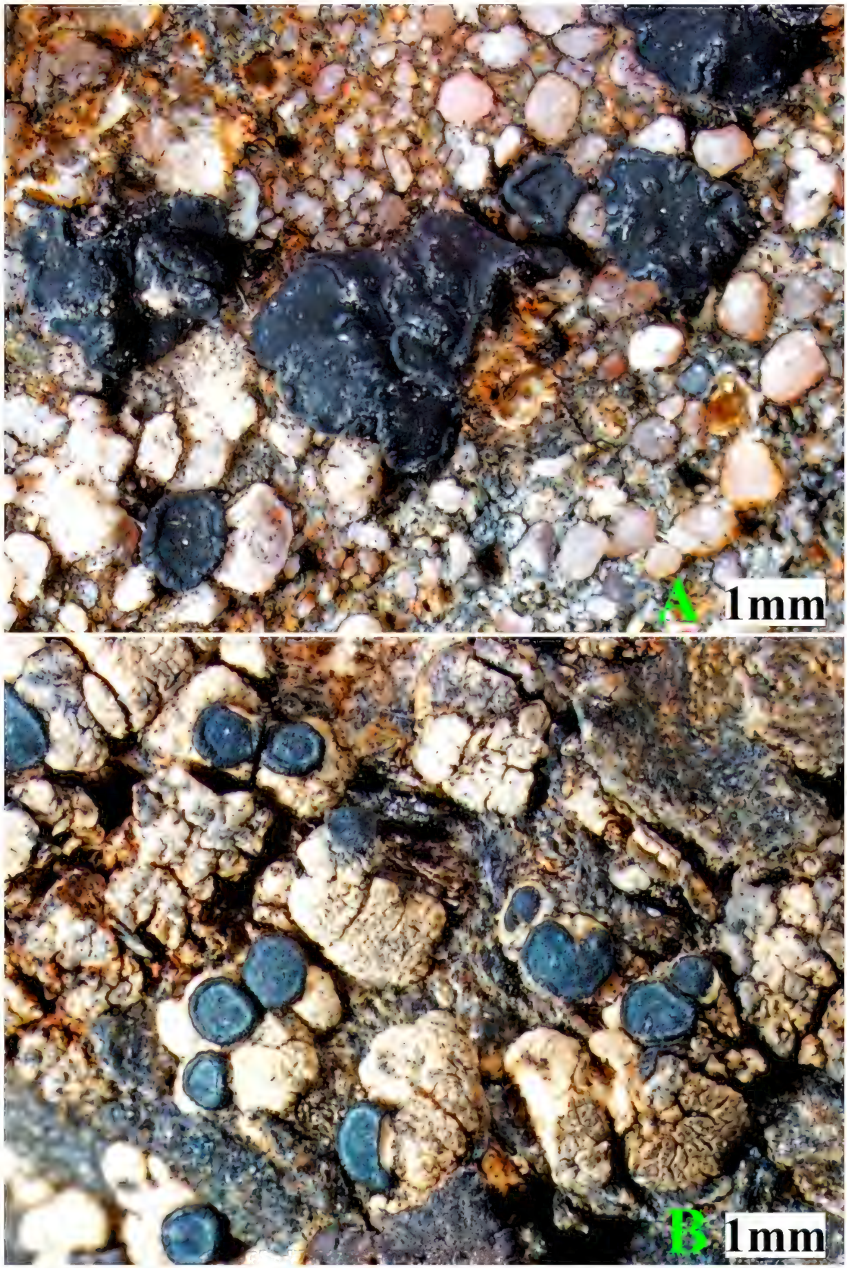

Fig. 2 A. *Lecidea promiscens* (L.S. Wang 00-19783, HKAS);
B. *Lecidella bullata* (J.C. Wei X024916, HMAS-L).

clavate, 8-spored; ascospores hyaline, simple, ellipsoid to broad-ellipsoid, 7–12 × 5–6 µm.

Spot tests — K+ yellow, C–, KC+ yellow, P+ yellow.

Secondary metabolites — atranorin, xanthones, ± psoromic acid, ± zeorin.

Specimens examined — **China. Yunnan:** Deqin county, Mt. Baimaxueshan, alt. 4500 m, on rock, 25 May 1985, L.S. Wang 8909(HKAS); **Xizang:** Mt. Qomolangma, Zhongrongbu, alt. 5550 m, on rock, 28 May 1966, J.C. Wei X024916 (HMAS-L).

Comments — *Lecidella bullata* is very similar to *L. stigmatea*, which also has a colorless internal excipulum and subhymenium. However, *L. stigmatea* has a very thin, rimulose or scattered verrucose thallus and no psoromic acid.

Reported from the Arctic, Europe, Asia, and North America (Inoue 1997, Thomson 1997), *L. bullata* is new to China.

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Puccinia, Uromyces, and *Xenodochus* species new to Turkey

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Abstract — The genus *Xenodochus* and nine rust taxa —*Xenodochus carbonarius*, *Puccinia hysteriiformis*, *P. majoricensis*, *P. pedunculata*, *P. stachydis*, *P. tendae*, *Uromyces colchici*, *U. kochiae, U. veratri*— are reported for the first time from Turkey. The morphological and microscopical features of these fungi are described.

Key words — Anatolia, Ardahan, Kars, *Pucciniales*, *Uredinales*

Introduction

The rust genus *Xenodochus*, with only seven species worldwide, differs from *Phragmidium* and *Frommeella* by teliospores having one germ pore in the apical cell and two or more germ pores in all other cells. *Puccinia,* the largest genus of *Uredinales* with about 3000 to 4000 species, has been recorded in all land areas except the polar regions.

In the last 10 years reports of new taxa and records of rust fungi have greatly increased in Turkey (Bahcecioglu & Gjærum 2003, 2004; Bahcecioglu & Yildiz 2005; Kabaktepe & Bahcecioglu 2005, 2006; Bahcecioglu et al. 2005, 2006, 2009; Kirbag 2003, 2004; Kirbag et al. 2011). Although approximately 350 rust species representing 26 genera have been recorded from Turkey, this number is far less than the total number of potential host plants, and further studies are required on rust fungi in Turkey.

This paper presents rust species collected from Kars and Ardahan provinces in northeast Turkey.

Materials & methods

Materials were collected during 2006–09 from Kars and Ardahan Provinces in Turkey. The host specimens were prepared according to established herbarium techniques. Spores were scraped from dried host specimens and mounted in lactophenol. The preparations were examined with an Olympus CX31 light microscope and micrographs taken with a Olympus E–330 camera. Analysis LS Starterwas software used to measure

at least 30 spores for each spore state. Host names follow Davis (1965–85) and Davis et al. (1988). Specimens are deposited in the Inonu University Herbarium (INU), Turkey.

Taxonomy

Xenodochus carbonarius Schltdl., Linnaea 1: 237 (1826)

Telia amphigenous, pulvinate, 1–2 mm diam, black. Teliospores elongate-cylindrical, often curved, 6–15 celled, rounded at the both ends, strongly constricted, smooth but subverruculose at the apex, 97–250 × 24–28 µm, each cell with 2 opposite superior pores, the uppermost with one apical pore with a small hyaline papilla, wall brown in basal cells often nearly colorless, 2 µm thick, pedicel short, colorless, persistent.

Known on *Sanguisorba officinalis* from Asia, Europe, and North America; *X. carbonarius* is a new rust species and *Xenodochus* a new genus for Turkey.

Specimens examined – On *Sanguisorba officinalis* L. (*Rosaceae*): **TURKEY: Ardahan**, 30 km from Cildir to Hanak, 1950 m, 10.07.2006, Sanli Kabaktepe 4109 (INU 8534); 20 km Cildir to Ardahan, 1880 m, 23.09.2006, Sanli Kabaktepe 4732 (INU 8535).

Puccinia hysteriiformis Peck, Bot. Gaz. 6: 276 (1881)

Telia amphigenous, scattered, covered by the epidermis, ≤ 1 mm diam, brown. Teliospores oblong or oblong-clavate, 40–45 × 19–23 µm, constricted at the septum, pores usually indistinct, pore of upper cell apical, pore in lower cell near the septum, wall 1.5–2.5 µm, at apex ≤ 4–9 µm, pale brown, smooth, pedicel ≤ 106 µm, persistent, hyaline.

Known from Europe, North America, and South America; *P. hysteriiformis* is a new rust species for Turkey.

Specimen examined – On *Minuartia recurva* (L.) Schinz & Thell. (*Caryophyllaceae*). **TURKEY: Kars**, Duman Mountain, 2000 m, 08.07.2006, Sanli Kabaktepe 4023 (INU 8537).

Puccinia majoricensis Maire, Bull. Soc. Myc. Fr. 21: 221 (1905)

Telia hypophyllous on stem and petioles, ≤ 1 mm diam, scattered or in groups, dark brown. Teliospores ellipsoid, oblong, 38–59 × 14–17 µm, constricted at the septum, pore of upper cell apical, wall 1–1.5 µm, at apex up to 5–8 µm, yellowish brown, at apex pale brown, smooth, pedicel ≤ 90 µm, persistent, pale yellow or hyaline.

Known from Asia and Europe, *P. majoricensis* is a new rust species for Turkey.

Specimens examined – On *Teucrium chamaedrys* L. (*Lamiaceae*). **TURKEY: Ardahan**, Posof, Dogrular village, 1600 m, 28. 08. 2008, Sanli Kabaktepe 6551(INU 8538); 13–15 km from Ardahan to Gole, 1850 m, Sanli Kabaktepe 6836 (INU 8539).

Remarks —*Puccinia annularis* (F. Strauss) G. Winter on *Teucrium chamaedrys* and *T. polium* L. (Bahcecioglu & Yildiz 2005; Kabaktepe & Bahcecioglu 2006;

Bahcecioglu et al. 2006) and *P. constricta* (Lagerh.) Bubák on *Teucrium orientale* L. (Bahcecioglu & Gjærum 2004) have also been reported from Turkey. *Puccinia majoricensis* differs from *P. annularis* by dark colored teliospores, and differs from *P. constricta* by a bigger and hyaline pedicel.

Puccinia pedunculata J.Schröt., Jber. schles. Ges. vaterl. Kultur 52: 117 (1875)

Uredinia hypophyllous, covered by the epidermis, roundest, scattered, reddish brown. Urediniospores ellipsoid, ovoid, 28–35 × 20–24 µm, wall 2.5–3 µm thick, pale brown, aculeate, 5–6 scattered pores. Telia hypophyllous, on stems, covered by the epidermis, dark brown. Teliospores ellipsoid, oblong or oblong-clavate, the upper cell broader, rounded or slightly attenuate, 41–58 × 20–26 µm, slightly or not constricted at the septum, pore of upper cell apical, wall smooth, chestnut brown, 1.5–2.5 µm, at apex 5–9 µm, pedicel ≤ 80 µm, persistent, brownish.

Known from Europe, *P. pedunculata* is a new rust species for Turkey.

Specimen examined – On *Rumex scutatus* L. (*Polygonaceae*). **TURKEY: Kars**, Sarikamis, Catak village, 1750 m, 24.08.2009, Sanli Kabaktepe 6934 (INU 8540).

Remarks — *Puccinia acetosae (*Schumach.) Korn. on *Rumex scutatus*, *R. tuberosus* L., and *R. acetosella* L. have been reported from Turkey. (Bahcecioglu & Yildiz 2005; Bahcecioglu et al. 2006; Tamer 1978). *Puccinia pedunculata* differs from *P. acetosae* by not being constricted at the septum and lacking a papilla on the upper teliospore cell.

Puccinia stachydis DC., Fl. franc. 2: 595 (1805)

Uredinia amphigenous, rounded, scattered, brown. Urediniospores ellipsoid, globoid, 25–28 × 24–26 µm, wall 2–3 µm thick, brown, aculeate with 2–3 pores. Telia similar to uredinia but dark colored. Teliospores ellipsoid or oblong, 37–47 × 19–23 µm, constricted at the septum, pore of upper cell apical, wall smooth, chestnut brown, ≤ 6–11 µm thick at the apex, pedicel as long as spores, persistent, hyaline.

Known from Europe, *P. stachydis* is a new rust species for Turkey.

Specimens examined – On: *Stachys annua* L. (*Lamiaceae*). **TURKEY: Ardahan**, 16–18 km Haskoy to Ardahan, 1850 m, 17.09.2008, Sanli Kabaktepe 6675 (INU 8541). **Kars**, Sarikamis, Catak village, 1750 m, 24.08.2009, Sanli Kabaktepe 6935 (INU 8542).; Sarikamis, 2100–2500 m, 25.08.2009, Sanli Kabaktepe 6996 (INU 8543).

Remarks —*Puccinia hariotii* Lagerh. on *Stachys* spp. (Henderson 1957) and *P. betonicae* (Alb. & Schwein.) DC. on *S. iberica* M. Bieb. and *S. citrina* Boiss. & Heldr. (Bahcecioglu & Yildiz 2005; Bahcecioglu & et al. 2006) have been reported from Turkey. *Puccinia stachydis* differs from *P. hariotii* by constriction at the septum and a persistent pedicel and from *P. betonicae* by pale colored teliospores and a persistent pedicel.

Puccinia tendae Gäum., Rev. Mycol. 18: 184 (1953)

Uredinia hypophyllous, rounded, scattered, pale brown. Urediniospores ellipsoid, globoid, 13–28 × 18–26 µm, wall 1.5 µm thick, brown, echinulate with 2–3 equatorial pores. Telia similar to uredinia but dark colored. Teliospores ellipsoid or oblong, 35–50 × 19–22 µm, constricted at the septum, pore of upper cell apical, wall smooth, pale chestnut brown, 2–3 µm thick, at apex up to 9 µm thick, pedicel ≤ 100 µm, persistent, hyaline.

Known from Europe, *P. tendae* is a new rust species for Turkey.

Specimen examined – On *Thymus fedtschenkoi* Ronniger (*Lamiaceae*). **TURKEY**: **Kars**, 8 km from Kagizman to Tuzluca, 1140 m, 12. 06. 2007, Sanli Kabaktepe 4989 (INU 8544).

Remarks —*Puccinia serpylli* Lindr. on *Thymus fallax* Fisch. & C.A. Mey., *T. fedtschenkoi,* and *T. kotschyanus* Boiss. & Hohen. (Henderson 1959, 1964; Bahcecioglu & Gjærum 2004; Bahcecioglu et al. 2006) has been reported from Turkey. *Puccinia tendae* differs from *P. serpylli* by narrow, dark colored teliospores.

Uromyces colchici Massee, Grevillea 21: 6 (1892)

Telia amphigenous, scattered, rather large, elliptical, sometimes circinate, up to 2 mm long, covered for some time by the epidermis, brown. Teliospores subgloboid to ovoid, rounded above with a flat hyaline papilla, 28–40 × 20–28 µm, smooth, pale brown, wall 2.5–3.5 µm thick, pedicel deciduous, hyaline.

Known from Europe, *U. colchici* is a new rust species for Turkey.

Specimen examined – On *Colchicum speciosum* Steven (*Colchicaceae*). **TURKEY**: **Ardahan**, Bulbilen Mountain, 2400 m, 18.07.2007, Sanli Kabaktepe 5423 (INU 8545).

Uromyces kochiae Syd. & P. Syd., Annls mycol. 10: 215 (1912)

Uredinia amphigenous, small, scattered, black. Urediniospores globoid, subgloboid, 21–25 × 21–24 µm, wall 1.5–2.5 µm thick, yellowish brown, echinulate with 6–9 pores. Telia similar to uredinia. Teliospores globoid, ellipsoid, 18–23 × 16–18 µm, wall smooth, brown, at apex dark colored, up to 3–5 µm thick at apex, pedicel ≤ 100 µm, persistent, hyaline.

Known from Asia and Europe, *U. kochiae* is a new rust species for Turkey.

Specimens examined – On *Kochia prostrata* (L.) Schrad. (*Chenopodiaceae*). **TURKEY**: **Kars**, 20 km from Digor to Igdir, 1500 m, 07.07.2006, Sanli Kabaktepe 3989 (INU 8546).; Kagizman, Esenkir village, 1220 m, 01.07.2008, Sanli Kabaktepe 6033 (INU 8547).

Uromyces veratri (DC.) J. Schröt., Jber. schles. Ges. vaterl. Kultur 49: 10 (1871)

Uredinia hypophyllous, rounded, scattered, chestnut brown. Urediniospores ellipsoid, 22–26 × 19–20 µm, wall 1–2 µm thick, pale brown, aculeate with 1–3 equatorial pores. Telia amphigenous, scattered, pulverulent, up to 2 mm long,

covered by the epidermis, blackish brown. Teliospores ellipsoid, ovoid, oblong, 22–30 × 14–18 µm, at apex with a flat hyaline papilla, wall smooth, brown, ≤ 7 µm thick at apex, pedicel deciduous, brownish.

Known from Asia and Europe, *U. veratri* is a new rust species for Turkey.

Specimen examined – On *Veratrum album* L. (*Melanthiaceae*). **TURKEY: Ardahan**, Posof, Ilgar mountain pass, 2540 m, 26.08.2009, Sanli Kabaktepe 7023 (INU 8548).

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Pseudobaeospora deckeri sp. nov.— a new agaric from central California

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Abstract — *Pseudobaeospora deckeri* is described from the central California coast. This new species is characterized by fruitbodies with deep purple to lilac tones, a pileipellis with irregularly shaped erect elements, an undifferentiated subpellis, absence of cheilocystidia, and strong alkaline-virescent reactions in the pileus subpellis and stipe context.

Key words — *Agaricales*, biodiversity, fungal taxonomy, Coast Redwood, Monterey Cypress

Introduction

The genus *Pseudobaeospora* Singer has recently undergone a generic revision, spurred by the recognition and description of many new taxa (Bas 2002, 2003). Following this revisitation, novel *Pseudobaeospora* species were discovered in Europe and California (Adamčík & Bas 2002, Desjardin 2004, Vellinga 2009). Two of the more recently described species are from the central coast of California: *P. stevensii* Desjardin (Desjardin 2004), from cypress and redwood habitats in Marin and San Mateo counties, and *P. aphana* Vellinga (Vellinga 2009), from Marin County. Considered with *Tricholoma microsporum* (Ellis) Sacc. (not yet formally assigned to *Pseudobaeospora*), these species represent the second and third *Pseudobaeospora* species reported from California and the fifth and sixth species reported from North America.

Following a cold precipitation event in March of 2009, many redwood-associated macrofungi (*Hygrocybe* spp., *Entoloma* spp.) fruited on the University of California Santa Cruz (UCSC) campus. While surveying this assemblage, I encountered a small purple agaric for which the generic disposition was not immediately obvious. While investigating the fruitbodies closely, I collected a range of representative specimens in January 2010, when the same mycelium produced over forty fruitbodies. In December 2010, while surveying macrofungi of the California coast with Erin Page Blanchard and Noah Siegel, I collected

specimens from two additional, relatively distant, sites on the central California coast. A striking member of the Central California mycobiota, *P. deckeri* represents the fourth described species of *Pseudobaeospora* in California and the seventh in North America.

Materials & methods

The macromorphology was described from fresh material. Micromorphological features were observed from both fresh and dried material. Standardized color notations were made using Kornerup & Wanscher (1984). Specimens were sectioned using a Nikon SMZ-10A dissecting microscope and mounted in 3% KOH with and without staining by aqueous Congo Red or Melzer's reagent. Micromorphological features were observed with a Nikon Eclipse E800 light microscope. Lamellae and lamellulae are denoted as L and l, respectively. Spore measurements are given with the arithmetic means denoted as $L_{avg} \times W_{avg}$. DNA was extracted with Sigma Extraction Kit- REDExtract-N-Amp Plant PCR using standard procedures; ITS1F and ITS4 primers were used for PCR reactions, and cleanup was performed with Exosap. Sequencing reactions used Big Dye v3.1 at 1/8 strength of manufacturer's instructions, on a 3730 Sequencer (Applied Biosystems, Foster City, California). The resulting sequence was edited and assembled with Sequencher 4.2.2 (Gene Codes Corp., Ann Arbor, Michigan) and deposited in Genbank (JF898319).

Taxonomy

Pseudobaeospora deckeri C.F. Schwarz, **sp. nov.** FIGS 1–2

MYCOBANK MB 561249

Similar to *Pseudobaeospora pyrifera* but pileipellis with erect hyphae, cap and stipe with slightly different colors and KOH reactions, and without cheilocystidia.

HOLOTYPE: **USA. CALIFORNIA**: SANTA CRUZ COUNTY, Santa Cruz, UC Santa Cruz, north side of Engineering 2 Building, 12 Jan 2010, CS 12Jan2010-1 (**Holotype** UCSC 7451; Genbank JF898319).

ETYMOLOGY: Named in honor of Lee Decker, instructor of biology and ocean science, whose passion has inspired generations of students.

FRUITBODIES collybioid, entirely rubbery-tough to fleshy and slightly cartilaginous when fresh, brittle in age, but rather persistent. PILEUS 10–27 mm wide, broadly convex and circular to plano-convex or uplifted and irregularly wavy; margin of young specimens distinctly but narrowly involute and in some specimens distinctly ribbed; very finely pruinose, evenly or in zones or blotches, especially near margin, in age nearly glabrous; deep royal purple (16E–F8) when young and moist, fading to dull brownish or grayish purple (14F4–6), sometimes in age with areas of obscure orange-brown tones (5D5), at margin paler to nearly white. Flesh thin, pallid lilac to darker purple just above lamellae. LAMELLAE L = 22–38(50), l = 3–5, subdistant to fairly close, adnate to finely and obscurely sinuate, ventricose in age, in some specimens intervenose and transvenose, when young dull gray (8B1–2) to dull lilac (13E3),

FIG 1. *Pseudobaeospora deckeri.*
Basidiocarps. NS 14Jan2010. Photo by Noah Siegel.

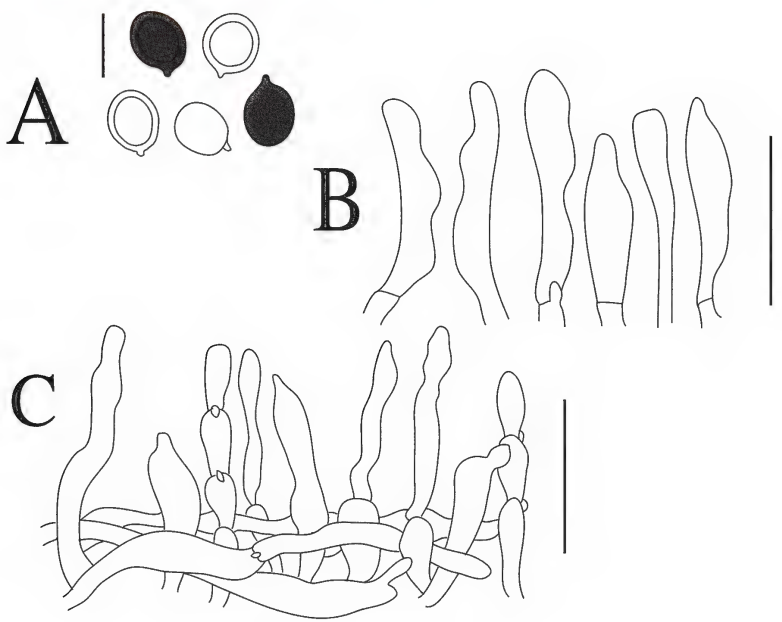

FIG 2. *Pseudobaeospora deckeri.*
A. Spores. B. Caulocystidia. C. Pileipellis. Bars: A = 3.5 µm, B–D = 30 µm.

in age distinctly ochre brownish (6C–D8); margin often thick, even to slightly irregular. Stipe (13–)29–50(–85) × 2–4.5 mm, equal or nearly so, at apex with belts or zones of fine, pale squamules, sometimes quite dense, with a fine pruina below these squamules over the upper quarter of the stipe, especially visible in young specimens, very dark purple (15F8) to royal purple (16D7–8) more or less remaining so at apex, but lower portions becoming duller purple to reddish-brown (11F7, 12D6, 12F4), at base usually conspicuously strigose, this tomentum white to lilac or purplish; base slightly rooting and bound to particles of substrate; flesh solid, dull, grayish purple, cartilaginous. Odor indistinct, although dried specimens wetted with alcohol emitted a 'mushroomy' odor (like *Agaricus bisporus*). Taste weakly but distinctly peppery or acrid. Macrochemical Reactions instantly dark blue-green in 3% KOH, this reaction obscured by the dark color of the fruitbodies and thus best detected by swabbing the treated fruitbody on white paper, or immersing a thin section in KOH. Spore Deposit white.

Basidiospores L × W = (2.8)3.6–3.8(4.8) × (2.8)3.3–3.5(3.8) µm. Q = (1.0) 1.05–1.1(1.3). A total of 40 spores from two fruitbodies were examined microscopically: i) (n = 20; from stipe apex, in Melzer's reagent) 2.8–3.8 × 2.8–4(4.8) µm, $L_{avg} \times W_{avg}$ = 3.6 × 3.3 µm, Q = 1.0–1.3, Q_{avg} = 1.1. ii) (n=20, from stipe apex, in Melzer's reagent) 2.9–4.3 × 2.9–3.8 µm, $L_{avg} \times W_{avg}$ = 3.7 × 3.5 µm, Q_{avg} = 1.05. Globose to subglobose or broadly ellipsoid, some with visible internal droplet; with conspicuous hilar appendage. Morphology and chemical characters of spores variable within a well defined range: immature spores smooth, thin or thick-walled and inconsistently dextrinoid, more mature spores dextrinoid within 5 minutes, thick-walled and smooth. Basidia 24–30 × 3.5–5 µm, clamped at base but obscurely so, tetrasporic, infrequently bisporic, protruding only slightly from lamellar face. Lamellar trama regular, composed of inflated elements 43–80 × 5–28 µm, barrel shaped and tapered at both ends, clamped. Walls irregularly and coarsely thickened. Cystidia absent, but irregularly shaped basidioles at the lamellar margin were inconsistently present. Pileipellis fundamentally a cutis of equal to slightly irregularly inflated hyphae 4.3–8.6 µm in diam., but with many erect elements, thus appearing as a trichoderm, remaining purplish in KOH; erect elements 16–58 × 3.6–6.7 µm, cylindrical to cylindrical-flexuous, often irregularly swollen, constricted or knobbed, often once or twice septate and clamped, often slightly entangled or clustered, more scattered away from disc, not encrusted. Subpellis of tightly interwoven hyphae, not or obscurely differentiated from pileal trama, distinctly green in KOH. Pileus trama parallel, made up of narrow, non-encrusted clamped hyphae. Stipitipellis at apex with a dense coating of clustered caulocystidia over a layer of narrow hyphae 2.8–5 µm in diam., attenuated over upper part of stipe. Caulocystidia 29–40 × 2.9–4.8 µm, cylindrical-flexuous

to strangulate or irregularly swollen and constricted or knobby, often once-septate and clamped; green in KOH (3%). Stipe Trama of inflated hyphae ≤ 8.6–14 µm in diam., pseudoparenchymatous in cross-section, strongly green in KOH. Clamp connections abundant in all tissues.

Ecology & distribution — Subcaespitose or scattered, terrestrial. Fruiting at the type locality amongst woodchip mulch in a landscaped area under coast redwood (*Sequoia sempervirens*). Currently known from three locations: the type locality (University of California Santa Cruz Campus, Santa Cruz Co.), Redwood Camp (Monterey Co.), and Skyline Community College (San Mateo Co.). At the southernmost known locality (Santa Cruz Co.) fruiting near huckleberry (*Vaccinium ovatum*), red alder (*Alnus rubra*), and coast redwood, while at the northernmost known locality (San Mateo Co.) fruiting in deep needle duff of Monterey cypress (*Hesperocyparis macrocarpa*). Fruiting dates include records from early December, January, and March.

Additional Collections examined: **USA. California**: Santa Cruz County, UC Santa Cruz, north side of Engineering 2 Building, 5 Mar 2009, CS 5Mar2009-7 (type mycelium); San mateo County, San Bruno, Skyline Community College, 14 Jan 2010, NS 14Jan2010-3; Monterey County, Big Sur, UC Big Creek Reserve, Redwood Camp, 13 Dec 2010, CS 13Dec2010-5.

Discussion

Pseudobaeospora deckeri is a small agaric that occurs throughout central California but probably has a wider distribution northwards. It is distinguished by its deep purple to violet pileus with a pale pinkish bloom, white spores, strong green KOH reactions, pileipellis with upright elements, frequent clamp connections, and lack of cheilocystidia.

Because of its deeply colored fruitbodies and KOH reactions, *Pseudobaeospora deckeri* shows affinity with *P. pyrifera* and *P. jamonii* in Bas' infrageneric *Pyrifera* and *Frieslandica* groups (Bas 2003). In Voto's worldwide key, *P. deckeri* could appear in either sect. *Anistoderma* Voto or sect. *Pseudobaeospora,* depending on interpretation of the pileipellis (Voto 2009). *Pseudobaeospora deckeri* can be distinguished from other members of the genus by its distinctly purple coloration combined with its alkaline-virescent reactions, a pileipellis that is an irregular cutis with abundant erect elements, abundant clamp connections, and a lack of a distinctly cellular subpellis and cheilocystidia.

In age, the fruitbodies resemble those of *P. stevensii*, with is sympatric at two of the known sites. Side-by-side comparison of such sympatric fruitbodies showed that *P. stevensii* possesses a 'yellower'-brown stipe with a coarser, paler, and generally more scurfy apex and a paler, more strongly strigose base, completely lacks violaceous tones, and shows an olive-green KOH reaction that is easily visible against the paler brown flesh. This contrasts with *P. deckeri*, in which the bluish-green color change in KOH is usually obscured by the purplish

flesh, and thus seeming blackish-vinaceous or blackish-olivaceous unless a thin section is made to reveal a solidly bright green color. Micromorphologically, *P. stevensii* has a cellular subpellis with no upright elements, cheilocystidia, and slightly more elongate spores with a higher average Q value.

Although photographs of *P. pyrifera* Bas & L.G. Krieglst. from Europe show a macroscopically similar entity, *P. pyrifera* has short broadly clavate cheilocystidia, a pileipellis of short, strongly inflated hyphae in chains, and a different KOH reaction in the stipe trama. Another European taxon, *P. jamonii* Bas et al., has a paler pileus, nearly free lamellae, and a pileipellis with scattered, broadly clavate erect elements amongst strongly inflated chains of hyphae (Bas et al. 2002). Also macroscopically similar is *P. dichroa* Bas, which has intense violet tones to the lamellae and stipe but a much paler pileus, only occasionally (then only slightly) intervenose lamellae, and a reddish-purple KOH reaction that eventually becomes yellowish-green. *Pseudobaeospora euganea* Voto has a browner pileus, a pileipellis with only scattered erect elements and a cellular subpellis, and significantly longer spores (Q = 1.14–1.45) (Voto 2009). *Pseudobaeospora cyanea* Arnolds et al. is a European species with a more intensely bluish-purple pileus, paler lamellae, a stipe with much less violet coloration, encrusted elements in the pileipellis, and significantly larger (4.0–5.5 µm × 3.3–4.0 µm) spores (Arnolds et al. 2003).

At least four other members of this genus (some undescribed) occur sympatrically, but all can be distinguished by their differently colored fruitbodies. *Pseudobaeospora aphana* produces much paler fruitbodies and smells distinctly fishy (Vellinga 2009); microscopically it is distinguished by the presence of cheilocystidia and a cellular subpellis. Species not known to occur in California include "*Pseudobaeospora pillodii*" sensu Redhead, *P. murrillii* E. Horak, *Tricholoma microsporum,* and *Agaricus fuscolilacinus* Peck, all of which lack KOH reactions and differ in other microscopic and macroscopic characters (Desjardin 2004).

The ITS sequence obtained from the type collection was aligned against that of *Pseudobaeospora pyrifera*; this comparison showed 93% identities (E-value = 0.0), clearly demonstrating generic placement but distinct species–level difference. Unfortunately, there are currently too few *Pseudobaeospora* sequences in GenBank to perform broader phylogenetic analyses.

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A new species of *Volvariella* from India

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Abstract—*Volvariella sathei* sp. nov., collected from Agharkar Research Institute Campus, Pune, is described, illustrated, and compared with morphologically closely related taxa of *Volvariella* and *Volvopluteus*. Diagnostic characters of the new species are the large white fruitbodies, relatively small basidiospores, and cylindric-clavate pleuro- and cheilocystidia. Neighbour-Joining analysis based on ITS sequences confirmed that it is distinct from its closest relative, *V. nivea* and other morphologically similar *Volvariella* species. A key to the Indian species of *Volvariella* and *Volvopluteus* is provided.

Key words—*Agaricales, Basidiomycetes*, macrofungi, taxonomy

Introduction

Volvariella Speg. (*Agaricales*) is well represented in India, and considerable work has been carried out by Pathak et al. (1978) and Pradeep et al. (1998), who have studied the genus and described and/or reported several species for the country. Agaric checklists compiled by Manjula (1983), Natarajan et al. (2005), and Kumaresan (unpublished list) cite 22 species of *Volvariella* including *Volvopluteus* Vizzini et al. as reported from India. A white agaric from Pune is here described as a new *Volvariella* species based on its morphological and molecular characters. Its phylogenetic position has been determined by ITS1+5.8S rDNA+ITS2 sequence analyses.

Materials & methods

Thin handmade sections were made from dried specimens, revived in 10% KOH, and stained in 2% Phloxine. The microscopical characters were studied using an Olympus U-CMAD3 microscope. Approximately 30 basidiospores from sections were measured; the range (extreme values in parentheses) precedes mean spore measurements (in parentheses). Camera lucida diagrams were made using Nikon E200 attached with Nikon Y-IDT prism. Colour terminology follows Kornerup & Wanscher (1978). The type specimen is deposited at Ajrekar Mycological Herbarium (AMH), MACS' Agharkar Research Institute, Pune, India.

Table 1. Sequences used in the phylogenetic analyses.

Species	Voucher No.	GenBank No.
Volvariella bombycina	AJ244	HM562212.1
V. caesiotincta	MA54717	HM562211.1
V. dunensis	SCM3513	JF415140.1
V. hypopithys	TO AV137	HM246492.1
V. lepiotospora	AJ155	HM562214.1
V. nivea	GDGM25489	FJ749127.1
V. nullicystidiata	SP393639	EU920671.1
V. pusilla	AJ51(LOU)	JF415137.1
V. pusilla	TO AV139	HM246494.1
V. sathei	AMH 9436	JN792550
V. strangulata	TO AV141	HM246493.1
V. surrecta	AJ55	HM562213.1
V. taylorii	AJ54	HM562210.1
V. terrea	LUG11010	JF415141.1
V. volvacea	TO AV143	HM246500.1
V. volvacea	ATCC MYA-4696*	HQ999973.1
Volvariella sp.	LOU18924	JF415139.1
Volvopluteus earlei (= V. acystidiata)	TO HG1973	HM246499.1
Vp. earlei (= V. cookei)	TO AV133	HM246496.1
Vp. earlei (≡ V. earlei)	TO AV134	HM246497.1
Vp. earlei (= V. media)	TO HG2001	HM246498.1
Vp. gloiocephalus (≡ V. gloiocephala)	TO AV136	HM246495.1
Schizophyllum commune	IFM 56967*	AB566277.1

*Strain no.

DNA isolation methodology followed Aljanabi & Martinez (1997) with slight modifications. ~300 mg of the holotype gills was washed thoroughly with 99% ethanol and water. DNA was extracted using high salt extraction buffer (NaCl, Tris. HCl and EDTA) and addition of one volume of phenol:chloroform:isoamyl alcohol (25:24:1) and finally precipitated with isopropanol, washed with 70% ethanol, and suspended in Tris-EDTA buffer. The protocol outlined by White et al. (1990) was followed for polymerase chain reaction in Eppendorf Master cycler (Eppendorf, Germany) with universal primers ITS5 and ITS4. Sequencing was performed using BigDye Terminator Cycle Sequencing Kit (Applied Biosystem, UK) as per manufacturer's instructions. The cleaned PCR product was run on ABI 3100 automated DNA sequencer. The raw sequences obtained from ABI sequencer were manually corrected for inconsistencies with the help of Chromas lite software. The DNA sequences obtained in Fasta format were subjected to BLASTn (http://www.ncbi.nlm.nih.gov) analysis.

For phylogenetic analysis, additional related sequences obtained from NCBI DNA sequence database (Table 1) were aligned by CLUSTAL W to generate a phylogenetic tree using MEGA v.5.0 software (Neighbour-joining using Kimura-2 parameter model). The outgroup comprised *Schizophyllum commune* and *Volvopluteus* spp. (recently transferred from *Volvariella*).

Plate 1. *Volvariella sathei*: Basidiomata under natural conditions in Agharkar Research Institute campus. A. Surface view of basidiome. B. Gill view of basidiome. Photo Senthilarasu G.

Taxonomy

Volvariella sathei Senthil., Rahul Sharma & S.K. Singh, **sp. nov.** PLATES 1–2

MYCOBANK MB 561949

Differs from *Volvariella nivea* in smaller subglobose to ovoid basidiospores and smaller pleuro- and cheilocystidia.

TYPE: India, Maharashtra State, Pune, Agharkar Research Institute Campus (18°52′N 73°83′E), on ground (soil), grass field, 03.08.2009, coll. G. Senthilarasu (**Holotype** AMH 9436; GenBank JN792550).

ETYMOLOGY: The epithet honors Dr. A.V. Sathe, a leading mycologist in western India.

PILEUS 8–9 cm diam., convex; surface white to yellowish white (4A2), fibrillose, shiny with fine radial striations in the margin; MARGIN thin, eroded; LAMELLAE free, remote, white becoming sordid, ≤ 10 mm wide, crowded with lamellulae of different lengths, margin concolorous; STIPE 80–105 × 9–13 mm, cylindric, distinctly compressed, tapering towards apex, solid; surface white to cream, glabrous; PILEAL CONTEXT 7 mm thick near stipe, white, unchanging on bruising; VOLVA free from the stipe, white (4A2), irregularly lobed, thick; ODOR fungus like; TASTE not recorded.

BASIDIOSPORES (5.5)6–6.5 × (4.5)5–5.5, (5.97±0.26 × 5.05±0.26) µm, Q = 1.1, subglobose to ovoid, hyaline with a stramineous thickened wall, smooth; BASIDIA 20–36 × 5.5–11 µm, cylindric clavate to clavate, tetrasporic; STERIGMATA short (≤ 2 µm), slender; LAMELLA EDGE heteromorphous; CHEILOCYSTIDIA scattered, 20–62 × 8–33 µm, cylindric-clavate with broadly rounded apex, thin-walled, easily collapsing; PLEUROCYSTIDIA scattered, 27–72 × 15–34 µm, cylindric-clavate, mucronate to broadly rounded apex, thin-walled; LAMELLA TRAMA convergent, consisting of thin walled, hyaline hyphae 5–18 µm diam.; SUBHYMENIUM little developed up to 20 µm wide, pseudoparenchymatous; PILEAL SURFACE a regular cutis consisting of radially repent to semi erect hyphae, thin-walled, 5–30 µm diam.; PILEAL CONTEXT consisting of tightly interwoven hyphae, 5–30 µm diam., thin-walled; VOLVA consisting of gelatinous layer, up to 50 µm thick, hyphae thin-walled, up to 33 µm diam., volval elements 20–47 × 15–24 µm, cylindric-clavate; STIPITIPELLIS hyphae made of cylindric, septate hyphae, 5–30 µm diam., terminal elements 30–45 × 12–13 µm, with rounded apex, caulocystidia absent; CLAMP-CONNECTIONS absent in all hyphae.

ADDITIONAL SPECIMENS EXAMINED: **INDIA, MAHARASHTRA STATE**, Pune, Agharkar Research Institute Campus (18°52′N 73°83′E), on ground (soil), grass field, 21.06.2009, coll. G. Senthilarasu (AMH 9443).

Justo et al. (2010, 2011) reassessed species traditionally assigned to *Pluteaceae* Kotl. & Pouzar based on molecular data. *Volvariella* has been placed outside the pluteoid clade with a new genus, *Volvopluteus,* erected to accommodate species with an ixocutis pileipellis and spores > 11 µm long. Consequently, *Volvariella gloiocephala*, *V. earlei,* and *V. acystidiata* have been transferred to

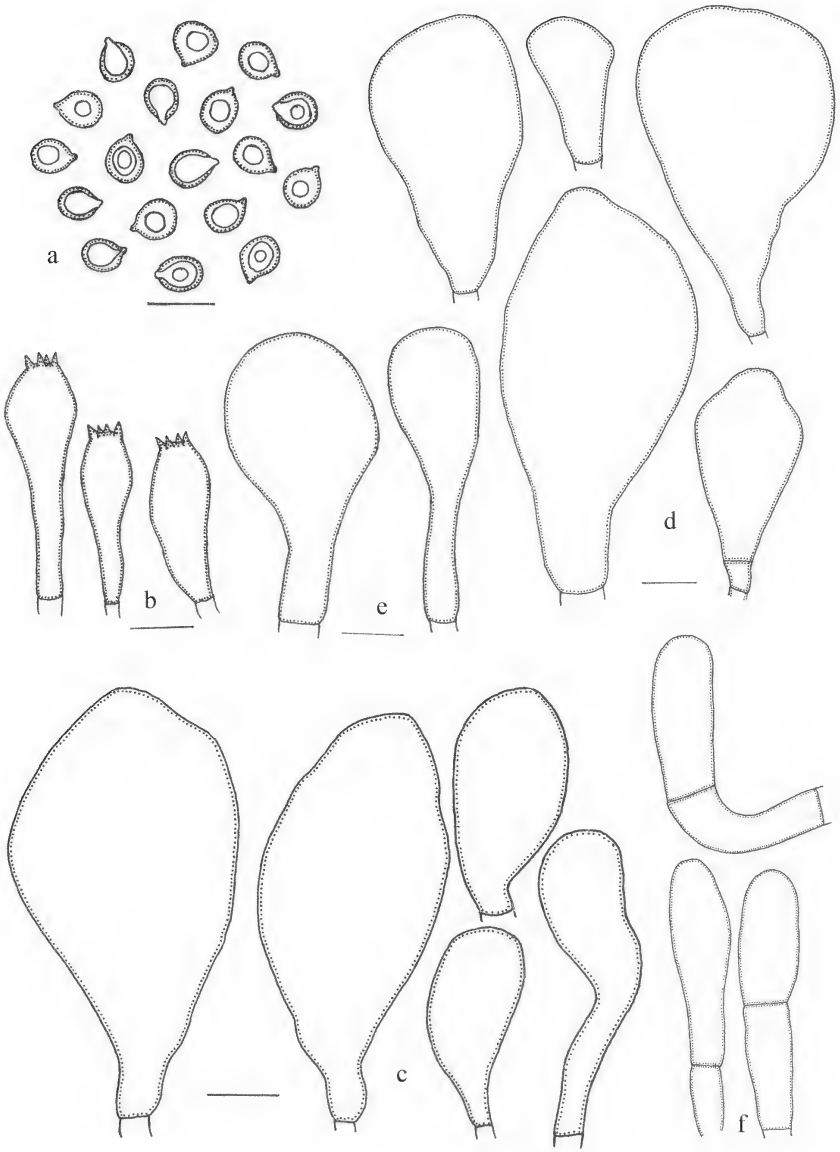

Plate 2. *Volvariella sathei*: a. Basidiospores. b. Basidia. c. Cheilocystidia. d. Pleurocystidia. e. Volval elements. f. Terminal elements of stipitipellis. Scale bar = 10 µm.

Volvopluteus. *Volvariella acystidiata* is considered now a form, as *Volvopluteus earlei* f. *acystidiatus* (N.C. Pathak) Vizzini & Contu (Justo et al. 2011).

Of the 20 species described from India, two belong in *Volvopluteus* (*Vp. gloiocephalus* (DC.) Vizzini et al. and *Vp. earlei* (Murrill) Vizzini et al.), and the remaining species belong in *Volvariella*. *Volvariella sathei* is compared here with morphologically similar taxa belonging to both genera.

Volvopluteus earlei, which also produces white basidiomata, differs in smaller (3–5 cm diam.) size and longer (>11 µm) spores (Justo & Castro 2010; Justo et al. 2010). *Volvopluteus earlei* f. *acystidiatus,* originally described by Pathak (1975) and later by Vizzini & Contu (2010), also closely resembles *V. sathei* but clearly differs by its smaller (20–30 mm diam.) yellowish tinged pileus with pinkish margin, shorter more slender stipe (30–40 × 2–3 mm), larger spores (10.5–16.5 × 7.5–10.5 µm) and basidia (45–70 × 10–13.5), and absence of hymenial cystidia (Justo et al. 2010). Phylogenetically *Volvopluteus* species are clearly distant from *Volvariella* species, including *V. sathei*. There are certain regions within ITS including 5.8S that distinguishes *Volvariella* from *Volvopluteus*.

Volvariella sathei is similar to *Volvariella pusilla* (Pers.) Singer (Pegler 1977, 1983, as *V. parvula*) in the white basidiomata with fibrillose pileus and remote lamellae. However, *V. pusilla* differs in stature with smaller pilei (1.5–3 cm diam.) and stipes (10–40 × 2–4 mm) as well as producing smaller basidia (17–29 × 5–7.5 µm), pleurocystidia (36–53 × 11–17 µm), and cheilocystidia (25–40 × 9–16 µm).

Volvariella nivea T.H. Li & Xiang L. Chen described from China (Li et al. 2009) also produces large, white basidiomata but clearly differs in slightly larger ovoid to broadly ellipsoid spores and considerably larger cheilo- (50–150 × 20–46 µm) and pleurocystidia (60–132 × 19–44 µm).

Volvariella bombycina (Schaeff.) Singer, *V. bombycina* var. *microspora* Dennis, *V. hypopithys* (Fr.) Shaffer, *V. liliputiana* (Henn.) G.C. Rath, and *V. delicatula* (Massee) Manjula are also similar to *V. sathei*. However, *V. bombycina* is lignicolous, and the spores are larger (7.3–9.4 µm in *V. bombycina*; 6–7.5 µm in *V. bombycina* var. *microspora*). *Volvariella hypopithys* has smaller basidiomes (pileus ≤ 5 cm diam.) with squamulose pilei and hairy stipes, while *Volvariella liliputiana* and *V. delicatula* are much smaller (pileus 5–30 mm diam.) than *V. sathei*.

A BLASTn search comparing the AMH 9436 530bp nucleotide sequences (comprising ITS1 = 210bp, 5.8S = 151bp, ITS2 = 157bp) with the Genbank DNA ITS sequence database showed maximum (84%) similarity with *V. terrea* (JF415141.1 query coverage 88%) and 83% similarity with *V. dunensis* (JF415140.1 query coverage 88%). Twenty-two ITS sequences representing 13 *Volvariella* spp., 2 *Volvopluteus* sp., and 1 *Schizophyllum* sp. obtained from Genbank database were aligned using CLUSTAL W with manual adjustments.

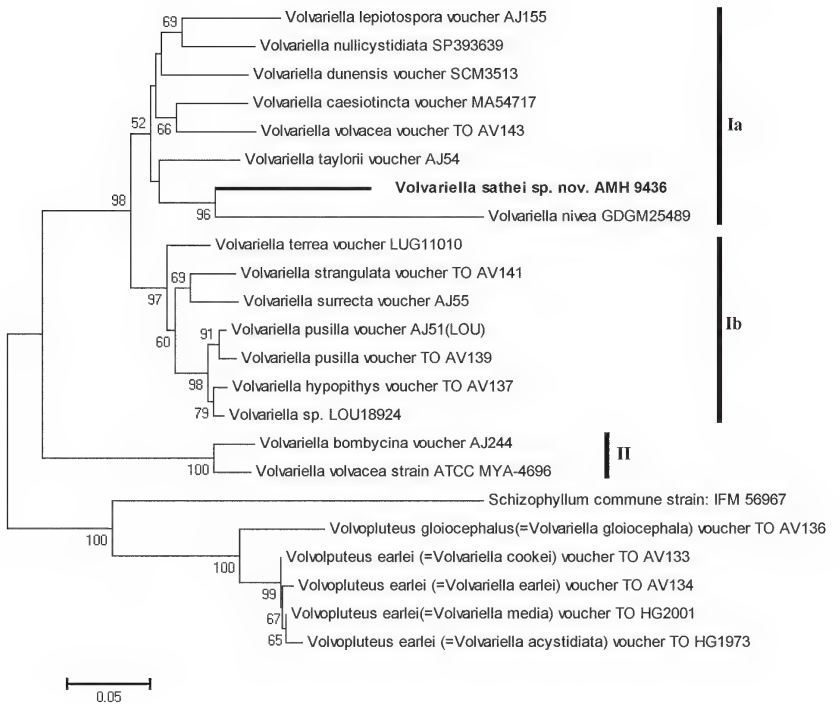
Volvariella lepiotospora voucher AJ155
Volvariella nullicystidiata SP393639
Volvariella dunensis voucher SCM3513
Volvariella caesiotincta voucher MA54717
Volvariella volvacea voucher TO AV143
Volvariella taylorii voucher AJ54
Volvariella sathei sp. nov. AMH 9436
Volvariella nivea GDGM25489
Volvariella terrea voucher LUG11010
Volvariella strangulata voucher TO AV141
Volvariella surrecta voucher AJ55
Volvariella pusilla voucher AJ51(LOU)
Volvariella pusilla voucher TO AV139
Volvariella hypopithys voucher TO AV137
Volvariella sp. LOU18924
Volvariella bombycina voucher AJ244
Volvariella volvacea strain ATCC MYA-4696
Schizophyllum commune strain: IFM 56967
Volvopluteus gloiocephalus(=Volvariella gloiocephala) voucher TO AV136
Volvolputeus earlei (=Volvariella cookei) voucher TO AV133
Volvopluteus earlei (=Volvariella earlei) voucher TO AV134
Volvopluteus earlei(=Volvariella media) voucher TO HG2001
Volvopluteus earlei (=Volvariella acystidiata) voucher TO HG1973
Ia
Ib
II
0.05

PLATE 3. Neighbour-joining tree showing phylogenetic position of *Volvariella sathei, Volvariella,* and *Volvopluteus* species. Bootstrap values > 50% are indicated.

The 5.8S region was almost perfectly aligned, but numerous gaps (indels) and misalignments among *Volvariella* species were observed in ITS1 and ITS2 regions. All analysed *Volvariella* species were divided into two main clusters (I and II, PLATE 3), with *V. sathei, V. nivea,* and *V. taylorii* together in subclade Ia. Although *V. sathei* and *V. nivea,* both characterized by pure white basidiocarps, are placed together with a strong (96%) bootstrap support, the branch lengths show considerable sequence divergence between them. Our analysis places the two sequences of *Volvariella volvacea* (HM246500.1, TO AV143; HQ999973.1, ATCC MYA-4696) far apart (in completely two different clusters), suggesting ambiguity in deposited sequences.

As Li et al. (2009) has indicated for *V. nivea*, we noted a high degree of variability (including considerable misalignment and several indels) throughout the ITS 1 and ITS 2 regions among *Volvariella* species, but the 5.8S region remained conserved with relatively few changes.

Key to species of *Volvopluteus (Vp.)* and *Volvariella (V.)* in India:

Two newly described species —*Volvariella indica* Pathak et al. (Manjula 1983) and *V. minuta* Kumar et al. (Kumar et al. 2009–10)— and a new record of *V. congolensis* N.C. Pathak (Kumar et al. 2009–10) from India are poorly known and have been excluded from the key.

1. Spores > 11 µm long, pileipellis is an ixocutis 2
1. Spores ≤ 10.5 µm long, pileipellis not an ixocutis 3

2. Pileus 5–15 cm diam.; spores 11–18 × 6.5–9.5 µm (ivory white to light brownish grey, often darker on the disc) ..... *Vp. gloiocephalus*
2. Pileus 3–5 cm diam.; spores 11–15.5 µm long ..... *Vp. earlei*

3. Lignicolous, on dead wood, pileus pale yellow then whitish 4
3. Growing on other substrata 7

4. Basidiome robust, > 5 cm diam; volva thick and fleshy 5
4. Basidiome small, < 5 cm diam; volva small, thin 6

5. Spores frequently 10–10.5 µm long ..... *V. diplasia*
5. Spores not > 9.5 µm long, volva white ..... *V. bombycina*

6. Volva internally rugoso-tomentose; lamellae pale flesh pink; spores 6–7 × 3–4 µm ..... *V. glandiformis*
6. Volva smooth; lamellae with brownish tints; spores 6.3–8 × 4–5 µm .... *V. thwaitesii*

7. Basidiome growing on other mushrooms ..... *V. surrecta*
7. Basidiome growing on soil or other organic substrates 8

8. Basidiome pure white to pinkish white 9
8. Basidiome ash green to brown 13

9. Pileus and stipe distinctly hairy (pileus ≤ 5 cm diam., white; spores 6–7 × 4.5–5 µm) ..... *V. hypopithys*
9. Basidiome not hairy, glabrous, subvelutinate to silky fibrillose 10

10. Pileus 8–9 cm diam. white, fibrillose; stipe 80–105 × 9–13 mm ..... *V. sathei*
10. Pileus small ≤ 3 cm diam.; stipe short and slender 11

11. Pileus ≤ 1 cm diam, subvelutinate; spores 4.5 × 4 µm ..... *V. liliputiana*
11. Pileus ≤ 3 cm diam., glabrous to silky fibrillose 12

12. Spores 5–6 × 3.2–4 µm, pileus at first pinkish white ..... *V. delicatula*
12. Spores 4.5–7.5 × 4–6.5 µm, pileus pure white to pale cream (silky smooth to silky fibrillose, volva white, appressed) ..... *V. pusilla*

13. Pileus ash-green to grayish brown, volva white ..... *V. woodrowiana*
13. Pileus different shades of brown 14

14. Volva, fleshy, rigid, pale to dark brown to blackish brown above, cracking into areolate squamules ..... *V. terastia*
14. Volva smooth, not squamose 15

15. Basidiome large and fleshy; pileus robust ≤ 12 cm diam., tawny brown to grayish brown; stipe 7–20 mm diam.; volva brown 16
15. Basidioma small to medium ≤ 6.5 cm diam.; stipe 2–6 mm diam.; volva grey, white or brown 18

16. Spores frequently > 9 µm long (6–10.4 × 4.5–7 µm) ..... *V. volvacea*
16. Spores not > 9 µm long. 17
17. Pileus light seal brown at the disc, paling to grayish vinaceous at the margin with fine brown radial striae; spores 4.5–8.6 × 3–5.3 µm ..... *V. cubensis*
17. Pileus bright chestnut brown, spores 5–6.5 × 3.5–4 µm ..... *V. castanea*
18. Pileus grayish; volva grayish 19
18. Pileus brownish; volva brown or white 20
19. Pileus evenly pale to dark grey, slightly darker at the disc; margin not striate ..... *V. taylorii*
19. Pileus evenly grayish with blackish disc; margin or the entire cap deeply grooved ..... *V. nigrodisca*
20. Pileus vinaceous brown at the disc, paler towards the margin; stipe very long, 4–10 cm × 3–6 mm; volva fuliginous brown ..... *V. pseudovolvacea*
20. Pileus ash gray to grayish brown with blackish radial fibrils; stipe short, 4–4.5 cm × 2–4 mm, volva white ..... *V. apalotricha*

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A new species of *Pleurophragmium* from India

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Abstract — A new anamorphic species, *Pleurophragmium indicum* (*Ascomycota*), is described from India and illustrated. This litter-inhabiting fungus was collected from dead *Dendrocalamus strictus* leaves and is characterized by ellipsoidal to obovoid, straight to curved, transversely 3-septate, brown conidia. A key is provided to all accepted *Pleurophragmium* species.

Key words — biodiversity, microfungi, taxonomy

Introduction

During our studies on taxonomy and diversity of microfungi associated with flowering plants of Western Ghat forests in southern India, a hitherto unknown anamorphic ascomycete with affinities to *Pleurophragmium* Costantin was recovered from moist-chamber incubated leaf litter of the bamboo, *Dendrocalamus strictus*.

Materials & methods

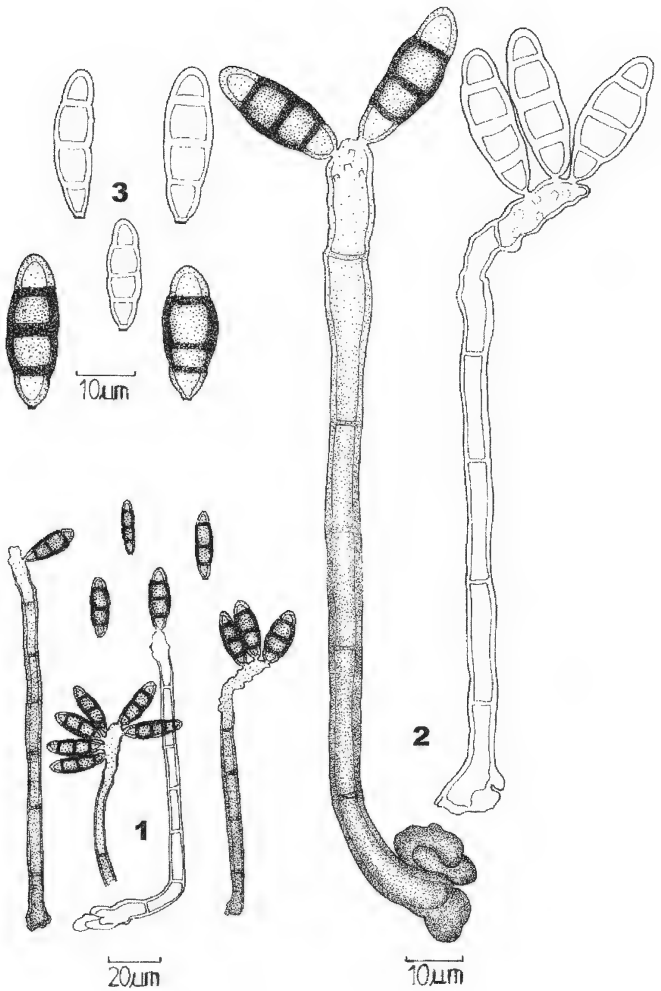
Dried and freshly fallen leaves of *Dendrocalamus strictus* were thoroughly washed in distilled water and incubated in a sterile moist chamber for one to several days at room temp (22–25°C). The leaves were examined at 2-day intervals under a stereoscope fitted with incidental light. Grey-brown colonies with erect to slightly flexuous conidiophores with a crown of conidia appeared on the surface of the leaves after 5–7 days of incubation. The fungus was mounted on a slide with lactophenol and examined under a bright field light microscope.

Taxonomy

Pleurophragmium indicum M.A. D'Souza & Bhat, **sp. nov.** Figs. 1–4

MycoBank MB563115

Differs from *P. bitunicatum* in conidia that lack a distinct outer covering and from *P. parvisporum* in its larger conidia and varied colouration.



Figs. 1–3. *Pleurophragmium indicum.*
1. Habit. 2. Magnified view of conidiophores with conidia. 3. Conidia.

Type: India, Molem Wildlife Sanctuary, Goa, on fallen dead and decaying leaves of *Dendrocalamus strictus* (Roxb.) Nees (*Poaceae*), 11 March 1999, leg. Maria A. D'Souza, (**Holotype**, GUBH 367).

Etymology: from the country of the type locality.

Colonies effuse, dark brown to black, velvety on the natural substrate. Mycelium partly immersed and partly superficial. Conidiophores mononematous, scattered, erect, straight to slightly flexuous, swollen and rhizome-like at the

FIG. 4. *Pleurophragmium indicum*. Conidiophores and conidia, 600×.

base, smooth, 4–6-septate, dark brown below, pale brown in the middle and above, thick-walled, 100–160 μm long, 7–22 μm wide at base, 4.5–6.5 μm wide in the middle and near apex. CONIDIOGENOUS CELLS polyblastic, integrated, terminal, cylindrical, sympodial, minutely denticulate, noncicatrized, smooth, pale brown to subhyaline, 15–23 × 4.5–9.5 μm. CONIDIA solitary, simple, dry, ellipsoidal to obovoid, rounded at the apex, narrower at the base, straight to slightly curved, smooth, transversely 3-septate, middle cells dark brown, end cells pale brown, 20–30 × 4.5–11 μm, 3–5 μm wide at the ends.

Discussion

The genus *Pleurophragmium*, with *P. bicolor* Costantin [= *P. simplex* (Berk. & Broome) S. Hughes] as type, includes 37 currently accepted taxa, with 27 other names placed in synonymy (Hughes 1958, Ellis 1971, 1976, Matsushima 1975, Kirk et al., 2008). Although the conidia are similar in shape and pigmentation to *Curvularia* Boedijn (Ellis 1971), *P. indicum* is differentiated by its blastic mode of conidial development.

Of those validly described species, *P. bitunicatum* Matsush., which resembles *P. indicum* in pigmentation and conidial size, differs by having conidia with distinct outer covering. *Pleurophragmium parvisporum* (Preuss) Hol.-Jech.

Table 1. Comparison of accepted species of *Pleurophragmium*

Species	Substrate	Type locality
P. acutum (Grove) M.B. Ellis 1976	Dead stems of *Urtica dioica*	England
P. angamosense Matsush. 1995	Decayed petiole of palm	Peru
P. aquaticum R.F. Castañeda et al., 2007	Decaying wood submerged in a stream	Mexico
P. arecae Matsush. 1987	Decaying leaves of *Areca catechu*	Taiwan
P. bicolor Matsush. 1975, nom. illegit.	Decayed leaves of *Quercus*, *Podocarpus*	Japan
P. bitunicatum Matsush. 1975	Decaying leaves	Japan
P. cylindrosporum Matsush. 1975	Decaying leaves	Japan
P. harunganae Hansf.1946	Pustules of *Hemileia* in leaves of *Harungana madagascariensis*	Uganda
P. hippotrichoides (Corda) M.B. Ellis 1976	Dead leaves, stems of *Cortaderia selloana*, *Umbilicus rupestris*, *Pandanus sylvestris*, and *Phormium tenax*	Europe, Channel Islands
P. indicum	Dead and decaying leaves of *Dendrocalamus strictus*	India
P. malaysianum Matsush. 1996	Decaying leaves	Malaysia
P. miniumbonatum (R.F. Castañeda et al.) R.F. Castañeda 2007	Fallen decaying leaves	Venezuela
P. naviculiforme Matsush. 1975	Decaying leaves	Japan
P. obcampanuloides Matsush. 1995	Decaying stems	Japan
P. parvisporum (Preuss) Hol.-Jech., 1972	Rotten leaves of *Musa sapientum*	Taiwan
P. peruamazonicum Matsush.1993	Decaying leaves of palm	Peru
P. peruamazonicum var. *inflatum* Matsush. 1993	Decaying and rotten leaves of palm	Peru
P. simplex (Berk.& Broome) S. Hughes 1958	Partly decorticated dead stems of *Urtica, Brassica, Polygonum*	Europe
P. subfusiforme Matsush. 1975	Decaying stems	Japan
P. triseptatum Matsush. 1975	Decaying stems	Japan
P. tritici M. B. Ellis 1976	Dead wheat culms	Ireland
P. varieseptatum Matsush. 1975	Decaying leaves of *Phyllostachys edulis*	Japan
P. verruculosum D.P. Tiwari 1970	Rhizosphere soil of *Piper betle*	India

produces similarly shaped conidia but differs in the uniformly pale ochraceous conidia that also are markedly smaller (14.5–25 × 4.5–6.5 µm).

Twenty-three accepted taxa of *Pleurophragmium* are compared (Table 1) and keyed out below.

Key to accepted taxa of *Pleurophragmium*

1. Conidia with 2 tunicae ..... *P. bitunicatum*
1a. Conidia without a tunica 2

2. Conidia smooth or indistinctly granular, 12–18 × 5–7µm ..... *P. harunganae*
2a. Conidia verrucose, finely verruculose or minutely echinulate 3
2b. Conidia smooth 4

3. Conidia verrucose, subclavate to clavate, 10–16.5 × 3.3–5 µm ..... *P. verruculosum*
3a. Conidia finely verruculose, ellipsoidal, 9–14 × 6–8 µm hyaline .. *P. hippotrichoides*
3b. Conidia minutely echinulate, ellipsoidal to fusiform, 6–11 × 3–4 µm ..... *P. tritici*

4. Conidia hyaline 5
4a. Conidia subhyaline to dematiaceous 6

5. Conidia naviculate, 6–12 × 2–3 µm ..... *P. naviculiforme*
5a. Conidia fusiform to ellipsoidal, 18–32 × 6.5–10 µm ..... *P. acutum*
5b. Conidia cylindro-clavate, (20 –) 40–75 × 4–5 µm ..... *P. malaysianum*

6. Conidia subhyaline 7
6a. Conidia dematiaceous 8

7. Conidia fusiform, curved, 3–7-septate, 21–38 × 5.8–9.2 µm ..... *P. subfusiforme*
7a. Conidia cylindrical, (1–)3(– 6)-septate, 14–25 × 4–5.7 µm ..... *P. triseptatum*
7b. Conidia fusiform to obclavate, (2–)3(–4)-septate, 12–24 × 3–4.5 µm ..... *P. arecae*

8. Conidia cylindrical 9
8a. Conidia of other shapes, not cylindrical 12

9. Conidia usually 3-septate 10
9a. Conidia 2– or 1–4-septate 11

10. Conidia pale to moderately brown, (15–) 20–28 × 2.8–4 µm
..... *P. bicolor* Matsush., nom. illegit.
10a. Conidia central cells brown, terminal cells pale brown, 25–40 × 5–6 µm
..... *P. cylindrosporum*

11. Conidia varicolored with a pale brown central cell and subhyaline terminal cells, 2-septate, 18–30 × 4–6 µm ..... *P. peruamazonicum*
11a. Conidia pale olive, 1–4-septate, 8–22 × 3–5 µm ..... *P. varieseptatum*

12. Conidia 1–2-septate 13
12a. Conidia 3 or more septate 14

13. Conidia obpyriform to turbinate, 1–2-septate, 11.5–15.5 × 5–6.5 µm .....
..... *P. obcampanuloides*
13a. Conidia ovoid, (1–) 2-septate, 17–28 × 8–1.5 µm
..... *P. peruamazonicum* var. *inflatum*

14. Conidia 5-septate, fusiform, slightly curved or straight, 24.5–40 × 5–7 µm ..... *P. angamosense*
14a. Conidia 3-septate, ellipsoidal to obovoid or fusiform to clavate 15
14b. Conidia 3–4-septate, obovoid, oblong, ellipsoidal to subclavate 16
15. Conidia ellipsoidal to obovoid, straight to curved, 20–30 × 3–11 µm ... *P. indicum*
15a. Conidia fusiform to clavate, sometimes navicular, 25–30 × 6.0–6.5 µm ..... *P. aquaticum*
16. Conidia ellipsoidal to subclavate, 10–21 × 3.5–6 µm ..... *P. simplex*
16a. Conidia obovoid, pyriform to broad clavate 17
17. Conidia obovoid, 10–25 × 4–6.5 µm ..... *P. parvisporum*
17a. Conidia obovoid, pyriform to broad clavate, umbonate at the apex, 16–19 × 6–7 µm ..... *P. miniumbonatum*

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New combinations in *Lactifluus*. 2. *L.* subg. *Gerardii*

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Abstract — In this second of a series of three papers, new combinations in the genus *Lactifluus* are proposed. This paper treats *Lactarius* subg. *Gerardii* (proposed here as new combination in *Lactifluus*). In this subgenus 17 combinations at species level are proposed.

Key words — milkcaps, nomenclature, *Lactarius*, *Russulaceae*

Introduction

This is the second treatment of species now classified in *Lactifluus*. *Lactifluus* subgenera *Edules*, *Lactariopsis*, and *Russulopsis* were discussed in the first article (Verbeken et al. 2012).

Taxonomy

Lactifluus subg. *Gerardii*

This recently established subgenus in *Lactarius* represents a group of milkcaps that is well defined both molecularly and morphologically. Species are agaricoid with brown pileus and stipe, contrasting pale lamellae, and a white spore print. Three deviant species —all three remarkably small, pleurotoid, and white *(Lactarius uyedae*, *L. conchatulus*, *L. genevievae*)— also belong to this subgenus. Their alliance with *Lactifluus* subg. *Gerardii* is confirmed by a multi-gene phylogeny but is also substantiated by their microscopic features. The ornamentation of the basidiospores in this subgenus is always reticulate and composed of interconnecting ridges, usually between 0.5 and 1.5 µm high. The pileipellis is typically a palisade with a strongly developed cellular subpellis. True pleurocystidia are rare. As of this writing, *L.* subg. *Gerardii*

contains 18 described species, all from North America, Asia, and the Australian region (Stubbe et al. 2012), and the group is absent from Africa and Europe. The type species, *Lactarius gerardii*, has already been recombined in *Lactifluus*. Stubbe et al. (2010) has demonstrated the existence of several cryptic species and species complexes in this subgenus, and the total number of species will likely increase after more data are gathered on several North-American milk-caps like *Lactarius atro-olivaceus*, *L. pseudogerardii*, *L. louisii*, *L. subtomentosus*, *L. subisabellinus* var. *murrillianus*, and *L. xanthydrorheus*.

Lactifluus* subg. *Gerardii (A.H. Sm. & Hesler) Stubbe, **comb. nov.**

MycoBank MB 564016

≡ *Lactarius* ser. *Gerardii* A.H. Sm. & Hesler, Brittonia 14: 378. 1962

≡ *Lactarius* subg. *Gerardii* (A.H. Sm. & Hesler) Stubbe, Fungal Biology 114: 280. 2010.

Type: *Lactarius gerardii* Peck

Lactifluus atrovelutinus (J.Z. Ying) X.H. Wang, **comb. nov.**

MycoBank MB 564017

≡ *Lactarius atrovelutinus* J.Z. Ying, Acta Mycol. Sin. 10: 191. 1991.

Lactifluus bicolor (Massee) Verbeken, **comb. nov.**

MycoBank MB 564018

≡ *Lactarius bicolor* Massee, Kew. Bull. Misc. Inf. 1914: 73. 1914.

Lactifluus conchatulus (Stubbe & H.T. Le) Stubbe, **comb. nov.**

MycoBank MB 564019

≡ *Lactarius conchatulus* Stubbe & H.T. Le, Fungal Diversity 52: 162. 2012.

Lactifluus coniculus (Stubbe & Verbeken) Verbeken, **comb. nov.**

MycoBank MB 564021

≡ *Lactarius coniculus* Stubbe & Verbeken, Fungal Diversity 52: 152. 2012.

Lactifluus genevievae (Stubbe & Verbeken) Stubbe, **comb. nov.**

MycoBank MB 564022

≡ *Lactarius genevievae* Stubbe & Verbeken, Fungal Diversity 52: 163. 2012.

Lactifluus gerardii (Peck) Kuntze, Revis. Gen. Pl. 2: 856. 1891.

≡ *Lactarius gerardii* Peck, Bull. Buffalo Soc. Nat. Sci. 1: 57. 1873, as "*geradii*".

Lactifluus hora (Stubbe & Verbeken) Stubbe, **comb. nov.**

MycoBank MB 564023

≡ *Lactarius hora* Stubbe & Verbeken, Fungal Diversity 52: 160. 2012.

Lactifluus leae (Stubbe & Verbeken) Verbeken, **comb. nov.**

MycoBank MB 564024

≡ *Lactarius leae* Stubbe & Verbeken, Fungal Diversity 52: 154. 2012.

Lactifluus leonardii (Stubbe & Verbeken) Stubbe, **comb. nov.**

MycoBank MB 564025

≡ *Lactarius leonardii* Stubbe & Verbeken, Fungal Diversity 52: 156. 2012.

Lactifluus limbatus (Stubbe & Verbeken) Stubbe, **comb. nov.**

MycoBank MB 564026

≡ *Lactarius limbatus* Stubbe & Verbeken, Fungal Diversity 52: 147. 2012.

Lactifluus ochrogalactus (Hashiya) X.H. Wang, **comb. nov.**

MycoBank MB 564027

≡ *Lactarius ochrogalactus* Hashiya, Mycoscience 47: 232. 2006.

Lactifluus petersenii (Hesler & A.H. Sm.) Stubbe, **comb. nov.**

MycoBank MB 564029

≡ *Lactarius petersenii* Hesler & A.H. Sm., N. Am. Species Lactarius: 106. 1979.

Lactifluus reticulatovenosus (Verbeken & E. Horak) Verbeken, **comb. nov.**

MycoBank MB 564030

≡ *Lactarius reticulatovenosus* Verbeken & E. Horak, Sydowia 53: 285. 2001.

Lactifluus sepiaceus (McNabb) Stubbe, **comb. nov.**

MycoBank MB 564031

≡ *Lactarius sepiaceus* McNabb, New Zealand J. Bot. 9: 50. 1971.

Lactifluus subgerardii (Hesler & A.H. Sm.) Stubbe, **comb. nov.**

MycoBank MB 564032

≡ *Lactarius subgerardii* Hesler & A.H. Sm., N. Am. Species Lactarius: 107. 1979.

Lactifluus uyedae (Singer) Verbeken, **comb. nov.**

MycoBank MB 564033

≡ *Lactarius uyedae* Singer, Nov. Hedw. 40: 436. 1985.

≡ *Pleurogala uyedae* (Singer) Redhead & Norvell, Mycotaxon 48: 377. 1993.

Lactifluus venosus (Verbeken & E. Horak) Verbeken, **comb. nov.**

MycoBank MB 564034

≡ *Lactarius venosus* Verbeken & E. Horak, Austr. Syst. Bot. 13: 692. 2000.

Lactifluus wirrabara (Grgur.) Stubbe, **comb. nov.**

MycoBank MB 564035

≡ *Lactarius wirrabara* Grgur., Larger Fungi S. Australia: 58. 1997.

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Stubbe D, Nuytinck J, Verbeken A. 2010. Critical assessment of the *Lactarius gerardii* species complex (*Russulales*). Fungal Biology 114: 271–283. http://dx.doi.org/10.1016/j.funbio.2010.01.008

Stubbe D., Le H.T., Wang X.-H., Nuytinck J., Van de Putte K., Verbeken A. 2012. The Australasian species of *Lactarius* subgenus *Gerardii* (*Russulales*). Fungal Diversity 52: 141–167. http://dx.doi.org/10.1007/s13225-011-0111-3

Verbeken A, Nuytinck J, Buyck B. 2012 ["2011"]. New combinations in *Lactifluus*. 1. *L.* subgenera *Edules*, *Lactariopsis*, and *Russulopsis*. Mycotaxon 118: 455–458. http://dx.doi.org/10.5248/118.447

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Book reviews and notices

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Introduction

The books reviewed here are as diverse as the fungi themselves, with molecular systematics of *Penicillium* and a field guide to the species of one genus in one country for conservation purposes as extremes of one continuum. Several publications stand out because of the high quality photos.

Book announcements include a volume on smut fungi in the series Flora Fungorum Sinicorum, a Spanish guide, and a Festschrift for the lichenologist Thomas H. Nash III.

Ascomycetes

Phylogenetic and taxonomic studies on the genera *Penicillium* and *Talaromyces*. Edited by R.A. Samson and J. Houbraken. 2011. Studies in Mycology no. 70. CBS-KNAW Fungal Biodiversity Centre, P.O. Box 85176, 3508 AD Utrecht, The Netherlands. <info@cbs.knaw.nl>. Pp. 183, illustr. ISBN 978-90-70351-87-8. Price: 60 € (paper copy), download free.

This work is significant in two respects. First, with a publication date of 15 November 2011, it is the first major work on the taxonomy of *Trichocomaceae* to have appeared since the separate nomenclatural status of anamorph-typified and teleomorph-typified names of pleomorphic fungi ended on 30 July 2011. Second, it is a significant contribution to the phylogenetic systematics of *Penicillium* and *Talaromyces* in its own right, presenting the results of four fresh studies.

Of particular importance is Houbraken & Samson's overview and revised phylogenetic system for not only penicillioid but also aspergilloid fungi. This

* Books for consideration for coverage in this column should be mailed to the Book Review Editor at the address above. All unsigned entries are by the Book Review Editor.

may come as a shock to some — with even two new families being recognized. Molecular phylogenetic studies using several gene show that three families are warranted: (1) *Aspergillaceae* for *Aspergillus* s. str. (syn. *Eurotium*, *Fenellia*, *Neocarpentales*, *Neosartorya*, other teleomorphs, and *Stilbothamnium*), *Hamigera* (incl. *Merimbla*), *Leiothecium*, *Monascus* (incl. *Basipetospora*), *Penicilliopsis, Penicillium* s. str. (syn. *Chromocleista*, *Citromyces, Eladia*, *Eupenicillium*, *Hemicarpentales*, *Thysanophora*, and *Toluromyces*), *Phialomyces*, *Phialosimplex*, *Polypaecilum*, *Sclerocleista*, *Warcupiella* (incl. *Raperia*), and *Xeromyces*; (2) *Thermoascaceae* for *Paecilomyces* (incl. *Byssochlamys*), and *Thermoascus* (incl. *Coonemeria*, and *Dactylomyces*); and (3) *Trichocomaceae* for *Rasamsonia*, *Sagenomella*, *Talaromyces* (incl. *Erythrogymnotheca*, and *Sagenoma*), *Thermomyces*, *Trichocoma,* and tentatively for *Dendrosphaera*. Four genera remained unplaced: *Ascorhiza*, *Dichlaena*, *Pseudocordyceps*, and *Sarophorum*. While some might think the authors "jumped-the-gun," as the names *Aspergillus* and *Penicillium* are such widely used names compared with those of the teleomorph-typified generic synonyms, the wording of the Melbourne CODE can be taken as indicating they were not obliged to have this approved by the General Committee on Nomenclature established by the Congress. In any event, there are no nomenclatural penalties for their choices, which follow the rules applying to all non-pleomorphic fungi. Following this revision of the familial and generic concepts and names, more detailed accounts of the subgenera and sections of *Penicillium*, with detailed lists of the species to be included in them, are presented.

This overview is followed by a critical revision of *Penicillium* sect. *Citrina* by Houbraken, Frisvad & Samson, based on molecular phylogeny and physiology (especially extrolites produced). Thirty-nine species are accepted, of which 17 are described as new. Extrolite profiles and calmodulin and β-tubulin sequences can be used for species separations, but only about half of the species could be unambiguously identified by ITS sequences alone. Rivera & Seifert address the *P. sclerotiorum* complex with an equally critical and polyphasic approach, recognizing seven species, of which three are newly described; in this case ITS barcodes satisfactorily separated the taxa. The final contribution by Samson and seven colleagues addresses *Talaromyces* (incl. *P.* subgen. *Biverticillium*) and considers all taxa described in the group; 69 species are accepted, one newly described, many new combinations are made from *Penicillium* into the genus, and a list of excluded names and synonyms is provided. As expected with Jens Frisvad as one of the co-authors, detailed information is given on the extrolites, with the "exometabolites" tabulated in detail.

All four contributions are superbly illustrated with coloured photomicrographs of colonies as well as fine shots of conidiophores and conidiogenous cells. This is a carefully and well-produced work, which is a "must" for all workers

on penicillioid fungi and their allies, and is sure to attract many citations. My one regret is that no culture or morphology based keys are included. This means that it is becoming increasingly impossible to identify fungi in this group precisely without molecular sequence data for several genes.

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Basidiomycetes

Riisikad. The genus *Lactarius* in Estonia. By K. Kalamees, 2010. Natural History Museum, University of Tartu. Available from Katrin Kolnes (katrin.kolnes@ut.ee). ISBN 978-9985-4-0619-9. Pp. 187, many col. pl. Price 21 €

This modern treatment of the genus *Lactarius* in Estonia includes a short introduction to the taxonomic placement of the genus, list of species recorded for Estonia, an ecology chapter, illustrated overview of the main characters for identification, and a key to the species. The key, which is illustrated, is in both Estonian and English. The main part of the book is taken up by the 60 species descriptions, with short descriptions of another 22 that might exist in the country. A glossary, list of references, and species index conclude the book.

Each species is presented by a good colour photo (often several) to illustrate colour variation and development. There are no microscopic details depicted. The text accompanying the photos covers sequence data, herbarium data, and ample ecological details. Original data are used throughout. A short English characterization of each species is very helpful for non-Estonian speakers.

The number of species is smaller than that treated in the work by Heilmann-Clausen et al. (1998) for northern Europe. But the extra information, the high quality photos, and the low price make this a good choice. In short, a well executed, well researched and very valuable contribution to our knowledge of milk caps.

Heilmann-Clausen J, Verbeken A, Vesterholt J. 1998. The genus *Lactarius*. Fungi of Northern Europe Vol. 2.

Släktet *Hygrophorus*, Skogsvaxskivlingar i Sverige. En fältguide till SMF:s svampväkteri "Vaxvakt". By E. Larsson, S. Jacobsson & A. Stridvall, 2011. Mykologiska Publikationer 3. Sveriges Mykologiska Förening, Institutionen för växt- och miljövetenskpaer, Göteborgs Universitetet, Box 461, 40530 Göteborg, Sweden. <www.svampar.se> . 56 pp.

In 2011 the Swedish Mycological Society started a project to record and monitor *Hygrophorus* species throughout Sweden. With only 15 species common and

well known, many species are currently considered vulnerable or under threat and included on the red data list.

To help identify the focus species, a small booklet has been produced offering species keys with descriptions and colour photos of all 37 taxa that enable field identification. The emphasis is on macroscopic characters, with spore dimensions as the only microscopic characters presented. Notes on ecology and distribution are given for each species, showing that many species are host tree specific, and some notes on possible confusion with other species complete the species information. There is also a recording sheet for the 15 rare species for which material should be sampled and sent to Ellen Larsson, the first author. Though written in Swedish, the booklet is easy to understand with some help from modern translation web sites and knowledge of another Germanic or Scandinavian language.

General

Systematics and evolution of fungi. By J.K. Misra, J.P. Tewari & S.K. Deshmukh (eds), 2012 ('2011'). PROGRESS IN MYCOLOGICAL RESEARCH Vol. 2. CRC Press, Taylor & Francis Group, 6000 Broken Sound Parkway, NW, Suite 300, Boca Raton, FL 33487, U.S.A. <www.crcpress.com> ISBN 978-1-57808-723-5. Pp. 412, Pl. 2. Price US$119.95.

The second book in a series of four on 'Progress in Mycological Research' focuses on systematics and phylogenetics, and 13 individual chapters treat various fungal groups.

An introduction by Hawksworth in the first chapter presents the merits and problems surrounding sequence-based systematics. Chapter 2 is devoted to fossil fungi, *Chytridiomycota* are treated by Powell and Letcher (Chapter 3), and Benny gives an overview of the *Zygomycota*, their systematics, and possible segregation into four groups (Chapter 4). Lichtwardt (Chapter 5) presents the evolution of the *Trichomycetes*, followed by Misra (Chapter 6), who covers the systematics of the two largest genera of the *Harpellales* (*Stachylina* and *Smittium*) along with keys and species descriptions. Chapter 7 focuses on two genera of edible mushrooms, *Morchella* and *Macrolepiota,* in Israel, and Barseghyan et al. try to delineate species based on ITS and EF-1alpha sequence analyses but describe no new species. Chapter 8 highlights morphological characters that distinguish groups of mushroom forming fungi, and authors Zmitrovich & Wasser warn that groupings based solely on RNA genes should be considered with skepticism. Tura et al. (chapter 9) gives an overview of *Phellinus* s.l. and *Inonotus* s.l. in Israel based on ITS sequences and morphology. In Chapter 10 Uli-Mattila, who discusses toxigenic *Fusarium* species from small cereal

grains, advocates a combined approach using all available characters for species recognition, while Gannibal (Chapter 11) presents the consequences of phylogenetic studies of alternarioid hyphomycetes. Tewari et al. (Chapter 12) shift gears to note methods for rapidly diagnosing *Candida*-related human pathogens. In the last chapter, Nagy et al. provide an overview of various phylogenetic methods and models to unravel the evolution in fungi.

Many articles are reviews and literature compilations, but some new research is presented. In all it gives an idea of the state of knowledge of the groups covered here.

Regional Floras

Fungi of tropical China. By X.-L. Wu, Y.-C. Dai, T.-H. Li, Z-L. Yang & B. Song, 2011. Science Press, Beijing, <www.sciencep.com>. ISBN-13: 9787030294708. Pp. 548, col. plates 495. Price circa US$ 220 [480.00 Yuan]

It is a pleasure to leaf through this book full of good mushroom photos from tropical China. In total 495 species are depicted, out of the 2065 listed for the area in the back. The book, authored by five mycologists and written in Chinese with Latin nomenclature and English chapter headings, gives an overview of the habitats (with their fungi), a checklist of all tropical fungi in China, and an extensive reference list; the main part of the book is devoted to species photos and descriptions. On the right hand page are two or three photos opposite the description on the even numbered pages. The majority of the taxa belong to the basidiomycetes. The species are ordered according to their phylogenetic position, for instance *Auriscalpium* can be found near the genus *Russula*. It is really nice to see so many ectomycorrhizal species depicted; *Amanita,* for instance, is well represented by 37 species and at least 35 species of boletes are included. Among the wood inhabiting fungi, *Ganoderma* stands out with 30 species, while stinkhorns in all their weird beautiful shapes and colours are represented by 21 species.

It is obvious that in some groups, names from species described from other regions have been applied to southeastern Asian specimens and are not always correct. Two such misapplied names are '*Lactarius deliciosus*' and '*Anthurus archeri,*' and the photo of *Suillus bovinus* does not look typical for that species.

Most species have been photographed in the field, but lab photos have been used for a very small number.

The book shows the diversity and richness of fungi in tropical China, and can certainly be used outside China. I encountered many taxa that are familiar from northern Thailand. I am looking forward to seeing an English translation of this book to make it more accessible to the rest of the world.

Mycota of Rhode Island: A checklist of the fungi recorded in Rhode Island (including lichens and myxomycetes). By R.D. Goos, 2010. THE BIOTA OF RHODE ISLAND vol. 4. Rhode Island Natural History Survey, P.O. Box 1858, Kingston, RI 02881, <programadmin@rinhs.org>. ISBN 1-887771-09-3. Pp. 228. Price US$60.00

Strangely enough, there do not seem to be many checklists of fungi for the U.S.A., either for separate states or as a whole. I tried to find such lists on line but was only able to find the database for plant pathogenic fungi at the U.S. National Fungus Collections. For European countries, checklists of macrofungi are seen as a prerequisite for recording and mapping programs, on which conservation efforts and red data lists can be based.

The present list for the state of Rhode Island, the smallest of the 50 states (comparable in size to the Cape Verde Islands), encompasses all fungi, from *Chytridiomycota* and *Glomeromycota* to *Basidiomycota;* slime molds and *Oomycota* are also covered. The list is restricted almost exclusively to verified records and based on a number of different sources (herbaria, literature). The presence of voucher specimens is a must.

A short history of the mycology practiced in the state forms the prelude to the main part of the book with the species lists. For each species the following information is given: full name including authority, host and substrate, an indication of distribution and rarity, and references and or herbarium records. The book is illustrated with nice line drawings by Roberta Calore.

It is great to have this information available, but of course this is the kind of data that should be available for all, and searchable in various ways on line.

BOOK ANNOUNCEMENTS

Biomonitoring, Ecology and Systematics of Lichens. Recognizing the Lichenological Legacy of Thomas H. Nash III on his 65th Birthday. By S.T. Bates, F. Bungartz, R. Lücking, M.A. Herrera-Campos & A. Zambrano (eds), 2011. BIBLIOTHECA LICHENOLOGICA Band 106. ISBN 978-3-443-58085-8. Pp. 442, col. pl. 16, figs 102. Price 109 €

Flora Fungorum Sinicorum Volume 39. *Tilletiales Urocystidales Entorrhizales Doassansiales Entylomatales Georgefischeriales*. By G. Lin, 2011. ISBN 9787030314536 Pp. 152 p., figs. Price around US$ 49.00.

Guía de hongos de la península ibérica. By G. Moreno & J.L. Manjón, 2011. Ediciones Omega, S.A. Calle Plató, 2608006 Barcelona, Spain. <www.ediciones-omega.es>. ISBN 978-84-282-1349-3. Pp. 1440, 590 col. pl. Price 100 €

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Regional annotated mycobiotas new to www.mycotaxon.com

Mycotaxon is pleased to announce the addition of five new species distribution lists to our "web-list" page at www.mycotaxon.com//resources/weblists.html, where a total of 89 checklist PDFs are now available for free download. The content and design of each list is the sole responsibility of its authors and their three (or more) expert reviewers. Those meeting Mycotaxon's scientific and nomenclatural criteria are accepted for posting. The authors, titles, and abstracts of our most recently accepted web-lists are provided below.

SOUTH AMERICA

Argentina

N. Niveiro & E. Albertó. Checklist of the Argentine *Agaricales* I. *Amanitaceae, Pluteaceae* and *Hygrophoraceae*. 24 p.

Abstract — A checklist of species belonging to families *Amanitaceae*, *Pluteaceae*, and *Hygrophoraceae* was made for Argentina. The list includes all species published till year 2011. Eleven genera and 150 species were recorded. The family *Pluteaceae* is the most cited for the country with 93 species.

Brazil

Isabella Pereira de Melo Wanderley Costa, Márcia Maria Costa Assunção, Thaís Emanuelle Feijó de Lima, Rafael José Vilela de Oliveira & Maria Auxiliadora de Queiroz Cavalcanti. Checklist of endophytic fungi from tropical regions. 26 p.

Abstract — Endophytes have great ecological and biotechnological importance and occur in a wide variety of plant families. This paper lists the genera and species of endophytic fungi from tropical regions with their hosts and references.

EUROPE

Czech & Slovak Republics

Alena Nováková, Alexandra Šimonovičová & Alena Kubátová. List of cultivable microfungi recorded from soils, soil related substrates and underground environment of the Czech and Slovak Republics. 186 p.

Abstract — A current list of the published microfungal records isolated from various soils and soil related substrates are presented from the territory of the Czech and Slovak Republics (formerly Czechoslovakia). Rhizosphere, entomopathogenic,

ovicidal, nematophagous, coprophilous, keratinophilic, dermatophytic, and thermoresistant fungi are also reported. The review contains microfungal species from peat and peat substrates, litter, vermicompost, enchytraeid and earthworm intestine and casts, and cattle environs, as well as records from soils affected by windthrow, fire, heavy metals and human activity. In addition, microfungi which are isolated from various substrates from underground environments, with the exception of airborne fungi, are included. A total of 1,332 microfungal records are reported in this list (54 of them belonging to yeasts or yeast-like microfungi and 1,278 belonging to filamentous fungi), representing 385 genera and 1,283 species of microscopic fungi. One microfungal taxon belongs to *Microsporidiomycota*, seven taxa belong to *Chromista*, 164 to *Mucoromycotina*, 1,133 to *Ascomycota*, and 28 to *Basidiomycota*. Forty-nine records were reported as undetermined fungi or sterile mycelia.

MID-EAST

Iran

Masoomeh Ghobad-Nejhad & Nils Hallenberg. Checklist of Iranian non-gilled/non-gasteroid hymenomycetes (*Agaricomycotina*). 41 p.

Abstract — This list includes 395 species of Iranian non-gilled/non-gasteroid hymenomycetes. Corticioid, poroid (polypores), hydnoid, clavarioid, cantharelloid, thelephoroid, and cyphelloid fungi are covered, together with heterobasidiomycetes. Almost all the species are supported by at least one herbarium specimen. The list has been compiled mainly from material collected by the authors, verified reports and literature. Nineteen species are reported for the first time from the country, and 118 species are added to the most recent account of aphyllophoroid fungi of North Iran by Hallenberg (1981, Mycotaxon 12: 473–502). The occurrence in Iran of six species is regarded as doubtful, while 12 species are excluded. The number of species known for each province in Iran is listed, with Golestan shown to be the most species-rich province. The total number of species recognized for major Iranian phytogeographical territories is also noted and *Funalia trogii*, *Inonotus hispidus*, and *Schizophyllum commune* are shown to be the most widespread species. A permanent repository for continuous updates has been made available via the Myco-Lich website, and future new records for the country will be published online. Users can access the latest changes to the checklist via subscription to a feed (RSS). The link to the checklist is http://www.myco-lich.com/mycology-of-iran/basidiomycota/checklists/National-checklist.

Turkey

Bahçecioğlu, Zeliha & Sanli Kabaktepe. Checklist of rust fungi in Turkey. 81 p.

Abstract — This study presents a checklist of rust fungi (*Uredinales*) of Turkey together with their known host plants. The checklist enumerates 351 species of rust fungi belonging to 26 genera and 9 families. Including 778 host plants belonging to 325 genera and 63 families. A host index is also provided.

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Minimizing the chaos following the loss of Article 59: Suggestions for a discussion

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Abstract — We assert that the suppression at the 2011 Melbourne Botanical Congress of the dual nomenclature for pleomorphic fungi was premature since most fungal genera still remain inadequately typified and need molecularly based revisions. The new system turns a rule-determined nomenclature into a system of names to be decided by committees. The new system cannot be implemented until experts publish well-documented rationales about the generic names to be accepted or suppressed among alternative morphs and the responsible committees act officially. Prioritization vs. suppression is not the same as conservation vs. rejection. If a teleomorphic generic name is suppressed, it still remains valid and legitimate and can still serve as a basis for names of higher-rank taxa. In most cases the teleomorph genus name should be prioritized unless convincing arguments favor preference of the anamorph name. Paraphyletic genera must be recognized. Unless the phylogenetic positions of all type species for possible morphs are confirmed, no prioritization can be fixed. When a binomial in a prioritized genus has a younger epithet than the corresponding name in the suppressed genus, only priority of extant names in the prioritized genus should count.

Key words — anamorph-teleomorph connection, competing morph names

Unification of fungal nomenclature (one name, whether teleomorphic or anamorphic, is allowed for each taxon) had to come eventually (Hawksworth et al. 2011, Norvell et al. 2011), but the move is being enforced much too early while only a minority of fungal taxa has been examined phylogenetically

(Hibbett et al. 2007). As currently circumscribed, too many fungal genera are unconfirmed as homogeneous and monophyletic (such proofs may not be possible for some genera), and knowledge of correlations between anamorph and teleomorph genera is far from comprehensive (Gams et al. 2011); however, Hawksworth et al. (2011) cited the ability to link anamorphs and their teleomorphs by molecular means as a key reason for the rapid adoption of the new rules. Names of lichenized fungi have always been excluded from dual nomenclature and are little affected by the issues discussed here (Lendemer 2011). While the previous Article 59 might not be reinstated (and cannot even be considered until the 2017 International Botanical Congress), we must find solutions to minimize the disruptive effects of this decision for fungal nomenclature and systematics. That the new procedures will depend on committee decisions listed in databases rather than on adherence to clear-cut rules of a Code will obviously be a source of conflicts.

A key sentence in Paragraph 1 of the Preamble in the ICBN (McNeill et al. 2006) is crucial to our argument: "This Code aims at the provision of a stable method of naming taxonomic groups, avoiding and rejecting the use of names which may cause error or ambiguity or throw science into confusion." This fundamental principle seems to be ignored by what has happened: Previous editions of the Code established democratically accepted rules subject to adjustment every six years by general voting in the Nomenclature Sessions. The newly prescribed rules and processes for determining the correct names for pleomorphic fungi reject these long understood, fair rules in favor of non-appealable decisions rendered by a series of Committees. It is still unknown how these newly adopted processes will be put into practice. Language adopted in Melbourne requests "consultation with" but does not actually mandate the involvement of non-IAPT committees (that have no legal standing vis-à-vis the Code). Nomenclatural stability, a principle long stated in the Code, seems to have been abandoned in favor of committee-based decisions governing the choices and applications of names for exactly those fungi whose complex biologies and multiple reproductive forms need governance by clear rules, not by consensus in committees.

Gene-based data can never be the sole basis for meaningful, informative classifications of any organism without also incorporating the essential foundations of morphology and other traditional characters. Imperfectly known taxa (new or old) need to be recollected for more study of their genes and morphologies before being reclassified. Fieldwork and ecological and morphological studies will be indispensible to the additions into GenBank of genetic data for innumerable neglected taxa. Deposits of accurate gene sequence data from incorrectly identified source organisms is a widespread problem that can confuse and seriously damage later analyses based on false assumptions.

While environmental DNA sampling might indicate that fungi are present, it reveals nothing about the appearance and biology of the fungi from which that DNA came, how to recognize them, or whether and how these fungi interact with any other organisms.

Some supporters of the one-fungus one-name (1F=1N) concept seem to assume that all fungi will readily yield good DNA and clean sequences. However, many entomophthoraleans resist repeated attempts by skilled technicians to obtain clean DNA and useful amplifications of genes now widely accepted (and expected) to provide phylogenetically informative data. Some taxa in the *Xylariaceae* yield either no cultures from too-scanty available materials or no useful PCR products from even freshly collected stromata. Further, some xylarioid stromata have at times provided contaminant DNA that was later shown to represent different taxa of the same or closely related xylariaceous genus (Stadler et al. 2010, Stadler 2011). Although *Xylariaceae* harbors few known endophytic species, some do grow hyperparasitically on xylarioid taxa; consequently, DNA extraction from stromata should be strongly discouraged, given that which organism provides the DNA sequence within a xylarioid stroma may remain uncertain. Endolichenic fungi may cause even more confusion (Arnold et al. 2009). As fungi generally grow in complex aggregations in nature, often only a skilled morphologist can ascertain that a culture actually represents what was thought to be the original inoculum. Some fungi may never be culturable unless new techniques are developed for isolation and growth, while special methods may also be needed to purify and to amplify their DNA. Anamorph-teleomorph relationships can hardly be proven in such cases, and phenotype-based taxonomic concepts MUST be maintained for the time being.

Implementation of the new rules

New paragraph approved in Melbourne:

> Art. 57.2 (**new**) In pleomorphic fungi (including lichenicolous fungi, but excluding lichen-forming fungi and those fungi traditionally associated with them taxonomically, e.g. *Mycocaliciaceae*), in cases where, prior to 1 January 2013, both teleomorph-typified and anamorph-typified names were widely used for a taxon, an anamorph-typified name that has priority **is not to be taken up** until retention of the teleomorph-typified name has been considered by the General Committee and rejected [OUR EMPHASIS].

This rule implies that the duality of names should not be suppressed until the responsible committees have rendered and publicized their decision about the preferential use of a particular name. Thus, mycological editors should be patient and tolerant with authors who may still prefer to use teleomorph-typified generic names, even if the corresponding anamorph name is older.

Prioritization—choosing between competing morph names for an individual pleomorphic fungus—vs. conservation.

New text passed in Melbourne to appear in the ICN:

> Art. 14.13 **(new)** In the interest of nomenclatural stability, for organisms treated as fungi (including lichenicolous fungi, but excluding lichen-forming fungi and those fungi traditionally associated with them taxonomically, e.g. *Mycocaliciaceae*), lists of names may be submitted to the General Committee, which will refer them to the Nomenclature Committee for Fungi (see Div. III) for examination by subcommittees established by that Committee in consultation with the General Committee and appropriate international bodies. Accepted names on these lists, which become Appendices of this CODE once reviewed and approved by the Nomenclature Committee for Fungi and the General Committee, are to be listed with their types together with those competing synonyms (including sanctioned names) against which they are treated as conserved (see also Art. 56.3).

PRIORITIZATION. We strongly suggest referring to the choice among competing morph names as **prioritization,** an action that is not equivalent to conservation. While Art. 14.13 requires such choices to be "treated as conserved," such status would confer permanent validity to frequently misapplied names.

To clarify this, Art. 14.13 should be amended to deal with suppressed names. Among competing anamorphic (occasionally synanamorphic) and teleomorphic names for the same taxon, one of the "name pair" will be given preference while the use of the other(s) is suppressed. Although suppressed names are not available for use, however, they remain nomenclaturally valid and legitimate. This condition differs fundamentally from the status of a NOMEN REJICIENDUM. In contrast to conservation, an act of prioritization need not be irreversibly fixed if convincing arguments support a change.

Consequently, names of teleomorphic genera that may be suppressed in favor of associated anamorphic generic names remain available as bases for HIGHER-RANK NAMES and would not disrupt the current general systematics of *Ascomycota* that would be severely affected by the issues discussed here.

Until July 31, 2011, teleomorph-typified names had **precedence** over anamorph-typified names irrespective of temporal priorities. Under the new rules, priority of publication should generally determine the choice. It is, however, true that the great majority of names for fungal families (and higher ranks) are based on teleomorph-typified genera. Because all teleotypified generic names remain legitimate and available as a basis for higher-rank taxon names, there is no objection to this (Art. 18.3 ICBN), and no changes at higher-rank names will become necessary for purely nomenclatural reasons. Basing any higher-rank names on anamorph-typified generic names might be desirable in some cases, but it must be discussed. If, for example, the name *Trichoderma*

were prioritized over *Hypocrea*, should this require introducing the names '*Trichodermataceae*,' '*Trichodermatales*,' etc.? Because family names adhere to the rule of priority, would the name '*Trichodermataceae*' have precedence over *Hypocreaceae* simply because of the priority of the genus name?

The new rule abandoning the preference of teleomorph-names for higher ranks of fungi may encourage the description of many higher taxa (families, orders, etc.) for clades of exclusively anamorphic *Ascomycota*. This would be a new — and undesirable — situation. Certain anamorph names have later been correlated with symplesiomorphic characters that have little or no use in formal taxonomy but do serve as descriptive terms.

For the prioritization of single or listed fungal names well-documented PROPOSALS must be submitted and published, e.g. in TAXON, as well as posted on an appropriate website. It might be helpful to first propose a **preliminary prioritization** that would become definitive only after a certain period of testing (e.g., after 2 years).

Decisions about prioritization will be made by **authorities** comprising a) a committee of experts (the largest, most diverse group reasonably possible), b) the International Committee for the Taxonomy of Fungi (ICTF), and c) the IBC Permanent Nomenclature Committee for Fungi (NCF). The NCF then will report to the General Committee. A majority of at least 60% of the votes (the NCF present practice—including abstentions, but suggested for (a) excluding abstentions) should be required for a decision. Art. 14.13 alludes to committees a) and b) as "appropriate international bodies." A primary concern is, who will bear the responsibility of establishing individual committees of experts?

The size and scope of any expert committee must vary according to the fungi under consideration to assure diverse points of view and geography; the constitution of these committees should and must be debated — and consensus reached — before they are established. The greatest problem will be finding mycologists competent in all fungal groups to be tackled. If enough experts are not found for a particular group or if no molecular analyses are available, then no prioritization of names should be decided. It will be a task of each expert committee's members to draft proposals of preferred names and vote on them. For example, *Hypocrea* vs. *Trichoderma* is being debated within the International Subcommission on *Trichoderma* and *Hypocrea* Taxonomy (ISTH); its outcome will be reported to the ICTF and NCF, who may (or may not) confirm it and publish their decision. In this case, the cellulase producer *Trichoderma reesei* and some other biocontrol strains of *Trichoderma* spp. are commercially important. Although the name of the *T. reesei* correlated teleomorph, *Hypocrea jecorina*, is also becoming widely used, preference would be given to *Trichoderma*, which is the older name anyhow. Does a preference for *Trichoderma* then force species

like *H. pulvinata* (in the anamorphically little-differentiated *Trichoderma* sect. *Hypocreanum*) and *H. danica*, *H. peltata*, *H. rhododendri*, *H. spinulosa*, etc. (no anamorphs) (Jaklitsch 2011) into *Trichoderma?*

The problem is that there are a) several groups of fungal taxa on which nobody is really working and b) other groups that are being well studied but by different authors with entirely incompatible concepts. How can a committee scenario work in these cases?

The implication that "expert groups" shall join in committees to treat their respective fields of specialty may pose serious problems. Taxonomic concepts in the *Eumycota* have always been highly variable (including even the name for all fungi). The introduction of molecular phylogenies raised the hope that such controversies might be resolved, but we now know this will not be the case. In fact, progress with molecular phylogenetic studies for diverse higher fungal taxa has been highly variable and depends on the variably patchy funding available to taxonomic specialists in different fungal groups. Sound molecular phylogenies are very difficult to achieve for innumerable fungal taxa for which most constituent species are neither epitypified nor available in cultures. Many who supported implementation of the 1F=1N concept seem not to have realized that morphological and other phenotypic data still provide more information on which to base classifications, especially where a limited number of sequenced genes fails to provide convincing proof of relatedness.

It is gravely worrying that TOO MANY new layers of authorities whose opinions may differ (and, indeed, be mutable) are injected into what was previously a process with universally recognized rules based on priority and an established line of authority to achieve stable, enduring nomenclatural decisions. The new system of committees adjudicating names EN MASSE means that research findings not favored by a committee can simply (and dangerously) be "voted out" of official recognition.

Suggested general working rules for committees

Names of genera

Anamorph and teleomorph genera were hitherto based on anamorph and teleomorph traits, respectively. Adoption of unitary nomenclature necessitates emending all pleomorphic generic diagnoses to recognize both anamorphic and teleomorphic characters, whether present or not. All mycologists should welcome such improvements because separating information about alternate morphs into multiple descriptions fails to communicate vital and complete information about the whole organism. Although we have often recognized some degree of polyphyly in many important anamorphic genera, we have until now preferred that teleomorphic genera be (as far as possible) monophyletic. The inverse situation may arise, however, when a more narrowly defined

anamorphic genus takes precedence over a teleomorphic one. In that case, the teleomorph genus may continue to be used as an artificial "dustbin" of teleomorph species with or without known anamorphs. For example, many *Mycosphaerella* anamorphs may be submerged into some morphologically and phylogenetically well-defined anamorphic genera such as *Cercospora* and *Ramularia* (Crous et al. 2009, Crous 2010), thus forcing *Mycosphaerella* to become a "dustbin" for species without known anamorphs. An equally undesirable alternative would be combining many hundreds of species from several anamorph genera into *Mycosphaerella*, which already contains ca. 2000 species. Morphological comparisons among species would become impossible in such a huge genus unless anamorph characters were correlated with phylogenetic ones. To avoid such problems, will we be forced to accept gigantic genera characterized only by statistics based on (minimal) DNA data?

If *Puccinia* were prioritized over *Uredo* s.str., the synonym *'Uredo'* would not be available for species with different teleomorphs, thus requiring the proposal of new generic names to accommodate them. Art. 14.13 may need to be amended in order to repair this undesirable situation.

Serious problems will arise for teleomorph genera such as *Cordyceps* and its recent segregates, each of which is associated with multiple distinct anamorph genera — many of which may form distinct clades and have names with nomenclatural priority over their linked teleomorph genus. Further splitting of (teleomorphic) genera may become advisable in such cases rather than lumping multiple disparate anamorphs within one teleomorphic genus.

This situation is exemplified by the relatively rare Asian teleomorphic genus *Metacordyceps* G.H. Sung et al. (*Hypocreales*: *Clavicipitaceae*) (Sung et al. 2007; also see Kepler et al. 2012) whose anamorphs include the globally distributed, commercially important entomopathogenic genera *Metarhizium* Sorokīn 1883 and *Nomuraea* Maubl. 1903, as well as *Pochonia* Bat. & O.M. Fonseca 1965 (including species used for biocontrol of nematodes) and other species not placed in any anamorphic genus after the phylogenetic reclassification of *Paecilomyces* sect. *Isarioidea* Samson 1974 (Luangsa-ard et al. 2005, Sung et al. 2007). That these anamorph genera are phylogenetically and ecologically distinct may justify the preferential use of the anamorph names. Nevertheless, the younger teleomorph name *Metacordyceps* also carries relevant information. Although we generally prefer to retain precedence for the teleomorph-typified generic name over its correlated anamorphic name, we must accept exceptions.

Exceptions to the precedence of teleomorph-generic names

Exceptions from this rule are allowed, although objective criteria are not yet clearly worded in the new Art. 57.2. To the *Trichoderma-Hypocrea* and *Metarhizium-Metacordyceps* examples noted above, we add the so-called

"macromycete" groups (those forming conspicuous stromata) in *Ascomycota* for possible exception.

Some *Hypocreales* (as in other ascomycete groups with economically important species, such as plant parasites or industrially cultivated fungi) have already been studied thoroughly by molecular methods. Taxonomic authorities familiar with these fungi will tend to follow the molecular results to adjudicate the status of the core taxa. However, their anamorph names for some of these fungi may prevail in future nomenclature, because the anamorphic stages that cause the conspicuous disease symptoms were described long before their corresponding teleomorphs. Nonetheless, nearly all xylarialean experts will admit that the number of taxa whose molecular characters have already been thoroughly evaluated is wholly insufficient to make final nomenclatural determinations, since the phylogenies based on housekeeping genes vs. rDNA present wholly divergent results about relationships among these fungi. The numerous experts (all also signatories of Gams et al. 2011) will surely agree that there is no need to change the current taxonomic system. Anamorphs of *Xylariales* are widely known but treated as synapomorphies, so that dual nomenclature was abandoned for these genera decades ago, whereas such well-known and conserved generic names as *Xylaria*, *Hypoxylon,* and *Daldinia* continue to take preference. Their anamorphic names are actually used as features that characterize subfamilies (or, in the future, perhaps, families or orders). The so far prevailing preference for teleomorph names could or should be adapted for all *Ascomycota* unless very serious reasons (including priority) make it more practical to adopt the anamorph name (as for *Penicillium* and anamorphs of *Metacordyceps*).

The latest revision of *Penicillium* (Samson et al. 2011) provides more examples: the teleomorphic name *Eupenicillium* has had little application or relevance, with *Penicillium* already treated as holomorphic by Raper & Thom (1948); however, for species of the more distantly related teleomorphic *Talaromyces* (whose anamorphs in *Penicillium* sect. *Biverticillium* still needed a new generic name), the teleomorph name was chosen to replace the better known anamorphic names in *Penicillium*. Must all these cases be decided again in the newly prescribed High Courts of Mycology by jurors yet to be selected?

In the future, teachers of mycology may be hard pressed to explain to their students why some genera of the *Xylariaceae* have nodulisporium-like or geniculosporium-like anamorphs but those of the "*Trichodermataceae*" (or "*Trichodermatales*") have *Hypocrea*-like teleomorphs. Recruitment of young fungal taxonomists is already very difficult; those who led the 1F=1N movement and championed the decisions adopted in Melbourne even used this difficulty as a major argument in their favor. Teaching of mycology MIGHT become more complex if suprageneric taxonomic ranks were often based on anamorphic

names, but such an argument seems to give little credit to the ability of students to understand these complications.

A further consequence of complex connections between anamorph and teleomorph genera is the necessary recognition of **paraphyletic genera,** such as *Lecanicillium*, which Gams & Zare (2001) described, at the same time recognizing its paraphyly. The current PCR-driven, monophyletic world view also seems to reject the perfectly valid, widely held position that paraphyletic taxa are not only acceptable, but that recognizing them as such is natural and appropriate. Zoologists, who have long accepted the reptiles as a transitional group from which the birds have arisen, do not deny *Reptilia* its own taxonomic status, even where both molecular and comparative anatomic studies prove that birds + reptiles are a paraphyletic class. It is hard to grasp why some mycologists fervently want to impose strict monophyly on all fungal taxa even where gene-based data supporting such a conclusion are unavailable or may be unobtainable) (Hörandl & Stuessy 2010).

When a new teleomorph is discovered for a known anamorph, it will no longer be possible to describe it in a new genus unless that species is proven phylogenetically not to be congeneric with the type of the anamorph genus.

If the type species of a genus has not been studied by gene-based methods, the genetic homogeneity of the genus cannot be proven. Similarly, no unification of anamorphs and teleomorphs can be forced if the type species of both the anamorph and teleomorph genera have not been analysed.

Names of species

If the rule of priority were strictly applied to epithets in both teleomorphic and anamorphic genera, many name changes would ensue unless a particular, well-established name is 'rescued' by formal conservation. To avoid this, we suggest a procedural rule for the committees (probably eventually for a new article in the Code) that binomials established before July 31, 2011 for either morph type must not be replaced by combining older epithets available in the 'opposite' morph (e.g., *Trichoderma reesei* should not become '*T. jecorinum*' and *T. citrinoviride* should not become '*T. schweinitzii*' despite the older available name *Hypocrea schweinitzii*). If *Pleospora* were retained over *Stemphylium*, would the new combination '*Pleospora botryosa*' then have to replace the established but younger *P. tarda*? In parallel with the ICBN Art. 11.2, such a rule would stipulate that priority applies only to names of the same rank or the same prioritized genus.

Another problem concerns species for which no sequence data are available. Can they be recombined into the alternative morph's genus or not? Would they remain in limbo? After proving the linkage of the type species of both anamorph and teleomorph genera, it would be better to combine all other names from the suppressed genus into the prioritized genus to keep them

visible and usable, even if those types were never restudied by any means. If not, they will be forgotten, any further revisions of them would halt, and homonyms or synonyms might be created in the prioritized genus.

Such issues are further complicated because innumerable older (and many highly important) species were not typified when described or their type specimens are lost; these taxa would require lecto-, neo-, and/or epitypifications before drawing any gene-based taxonomic conclusions about their status. The problems raised by so many fungi that were described from single collections and that may be extremely rare or difficult to re-collect again for DNA-based studies are nothing less than a daunting impediment for the establishment of a workable 1F=1N taxonomy.

Conclusions

A major aim of the one-fungus one-name policy was to simplify fungal nomenclature. The question, however, remains: simplification for whom? For the convenience of present-day mycologists, it would be desirable not to change the names of higher taxa. When in extreme cases an anamorph-generic name replaces the type genus of teleomorph-based higher taxa (e.g., *Hypocreales*, *Hypocreaceae*), we still remember the meaning of the higher taxon because we are used to it. The situation becomes complicated for future generations, however, who must deal with many previously teleomorph-based higher taxa when the teleomorph genus is no longer in use; mycologists must then learn that these names were based on a now-rejected nomenclatural practice and try to understand the superseded taxonomies BEFORE they can apply the systematic concepts that led to the current use of a name. The intended "simplification" imposed by the 1F=1N standard will thus not be achieved in a long-term vision, and future mycologists will still need to understand the suppressed dual nomenclature to deal with the historical literature. Moreover, many recently introduced teleomorph-generic names do reflect improved phylogenetic knowledge, although they will not successfully compete with the correlated anamorph names; these names undoubtedly will retain their informative value in naming clades and should not, therefore, be completely eliminated.

In a carefully balanced system of prioritization, future mycologists may appreciate having the choice between either continuing to use the present higher-rank taxon names or creating new anamorph-based names.

An essential, permanent remnant of the dual nomenclature will be the need for listing all teleomorphic and anamorphic synonyms of a fungus in any monographic treatment together with the typifications of all these names. It will often be the case that more species were described under the suppressed morph-generic name than in the officially recognized morph genus. In any case, all these suppressed species will have to be re-studied before any new species can

be described. This does not simplify future taxonomic work. Re-examination of types must be possible any time, and this will involve morphological (including ultrastructural), biochemical, and molecular approaches equally, all still subject to improvement. Curiously, it is often more valuable to preserve teleomorphic material of most ascomycetes in a herbarium than their anamorph which, if the type is a dried agar culture, can display its properties only when reactivated in culture.

In a unified nomenclature, fungi will continue to have complicated life cycles displaying a teleomorph and one or more anamorphs, each with its distinctive gene expression and biological and ecological role. Future students of mycology will have to understand the concept of anamorph and teleomorph to understand the biology of the fungus, even if all stages are encompassed under a single name.

The first paragraph of the Preamble of the ICBN contains an odd and ominously predictive stipulation immediately before the sentence quoted above: "The purpose of giving a name to a taxonomic group is **not to indicate its characters or history** [our emphasis added], but to supply a means of referring to it and to indicate its taxonomic rank." It seems probable that few mycologists who deal with pleomorphic fungi would appreciate the strict application of such a stipulation. The nomenclaturally required consolidation of many morphologically disparate teleomorphic and anamorphic forms and even genera into single genera now required by the newly adopted standard of 1F=1N may satisfy both the new Code and advocates of strict monophyleticism but this process will create many significantly large and diverse — but nomenclaturally supported! — genera that will also be self-accreting mycological 'black holes'. The names of such 'megataxa' will bear no useful biological information about their species. While this result will conform with the newly redefined (if artificial) sense of nomenclatural stability, it cannot be hailed as progress toward biological or even taxonomic sensibility.

We should also note that the availability of cultures of hitherto unknown fungal organisms is an absolute prerequisite for their future exploitation in biotechnological applications. Fungi have historically been used for many beneficial purposes; curiously, the roots for the new concepts and changes adopted in Melbourne seem to have been elaborated mainly for organisms that were studied intensively for their diverse beneficial or detrimental properties — e.g., pathogenicity, production of mycotoxins or other primary or secondary gene products, uses as biological control agents, or many other potential concerns. We should realize that in view of future applications, we have so far only dealt with the low-hanging fruits. Large-scale bioprospecting programs are now under way, and potential uses for fungi are by no means restricted to the traditional search for novel, badly needed antibiotics and other compounds

with potential utility in pharmaceutical or agrochemical indications. The search for industrial enzymes and commodity chemicals for "White Biotechnology", as well as renewable resources (biofuel production), bioremediation, etc., will almost certainly become more important in the future. Innovative methods of genomics research will allow for the exploitation of slow-growing organisms, e.g., by transfer of their genes into heterologous hosts where biotechnological applications appear feasible.

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Volume 119, pp. 509–511 *January–March 2012*

Nomenclatural novelties and typifications proposed in Mycotaxon 119

Abrachium Baseia & T.S. Cabral, p. 424

Abrachium floriforme (Baseia & Calonge) Baseia & T.S. Cabral, p. 424

Acarospora gallica H. Magn. 1929, neotypified, p. 374

Alveariospora Meir. Silva, R.F. Castañeda, O.L. Pereira & R.W. Barreto, p. 110

Alveariospora distoseptata Meir. Silva, R.F. Castañeda, O.L. Pereira & R.W. Barreto, p. 110

Anaseptoidium R.F. Castañeda, Heredia & R.M. Arias, p. 141

Anaseptoidium mycophilum R.F. Castañeda, Heredia & R.M. Arias, p. 142

Antrodia madronae Vlasák & Ryvarden, p. 220

Ceriporiopsis pseudoplacenta Vlasák & Ryvarden, p. 222

Coltricia indica Baltazar, p. 386

≡ *Coltricia pusilla* J.R. Sharma & J.E. Wright 1992 ['1989'], nom. illeg.

≡ *Coltricia parvula* J.R. Sharma & J.E. Wright 1997, nom. illeg.

Corynesporella obclavata L.G. Ma & X.G. Zhang, p. 83

Cylindrosympodium sosae R.F. Castañeda, Heredia & R.M. Arias, p. 145

Deconica deconicoides (E. Horak, Guzmán & Desjardin) Guzmán, p. 67

Diorygma agumbense B.O. Sharma & Khadilkar, p. 2

Diorygma dandeliense B.O. Sharma & Khadilkar, p. 3

Diorygma karnatakense B.O. Sharma & Khadilkar, p. 4

Diorygma rupicola B.O. Sharma & Makhija, p. 5

Endophragmiella gardeniae Jian Ma & X.G. Zhang, p. 104

Endophragmiella machili Jian Ma & X.G. Zhang, p. 105

Fulvifomes cambodiensis L.W. Zhou & W.M. Zhang, p. 176

Fuscoporia palomari Vlasák & Ryvarden, p. 218

Gymnopus inusitatus var. *cystidiatus* Antonín, Finy & Tomšovský, p. 294

Helvella fusca Gillet (1879), lectotypified & epitypified, p. 434

Herpothallon weii Y.L. Cheng & H.Y. Wang, p. 440

Heteroconium annesleae S.C. Ren & X.G. Zhang, p. 363
Heteroconium neolitseae S.C. Ren & X.G. Zhang, p. 363
Hyphoderma bicystidiatum Priyanka & Dhingra, p. 255
Hyphoderma subglobosum Priyanka & Dhingra, p. 257
Hysterostomella connari Inácio, Pereira-Carvalho, E.S.C. Souza, H.B. Sales & Dianese, p. 308
Ijuhya oenanthicola Lechat & Hairaud, p. 249
Intraornatosporaceae B.T. Goto & Oehl, p. 121
Intraornatospora B.T. Goto, Oehl & G.A. Silva, p. 122
Intraornatospora intraornata (B.T. Goto & Oehl) B.T. Goto, Oehl & G.A. Silva, p. 122
Lactifluus subg. *Gerardii* (A.H. Sm. & Hesler) Stubbe, p. 484
Lactifluus atrovelutinus (J.Z. Ying) X.H. Wang, p. 484
Lactifluus bicolor (Massee) Verbeken, p. 484
Lactifluus conchatulus (Stubbe & H.T. Le) Stubbe, p. 484
Lactifluus coniculus (Stubbe & Verbeken) Verbeken, p. 484
Lactifluus genevievae (Stubbe & Verbeken) Stubbe, p. 484
Lactifluus hora (Stubbe & Verbeken) Stubbe, p. 484
Lactifluus leae (Stubbe & Verbeken) Verbeken, p. 484
Lactifluus leonardii (Stubbe & Verbeken) Stubbe, p. 484
Lactifluus limbatus (Stubbe & Verbeken) Stubbe, p. 485
Lactifluus ochrogalactus (Hashiya) X.H. Wang, p. 485
Lactifluus petersenii (Hesler & A.H. Sm.) Stubbe, p. 485
Lactifluus reticulatovenosus (Verbeken & E. Horak) Verbeken, p. 485
Lactifluus sepiaceus (McNabb) Stubbe, p. 485
Lactifluus subgerardii (Hesler & A.H. Sm.) Stubbe, p. 485
Lactifluus uyedae (Singer) Verbeken, p. 485
Lactifluus venosus (Verbeken & E. Horak) Verbeken, p. 485
Lactifluus wirrabara (Grgur.) Stubbe, p. 485
Lecanora expallens Ach. (1810), lectotypified, p. 198
Lecanora expallens var. *conizaea* Ach. (1810), lectotypified, p. 198
Leptogium weii H.J. Liu & S. Guan, p. 413
Marasmiellus milicae Mešić, Tkalčec & Antonín, p. 234
Mycena moconensis Niveiro, Albertó & Desjardin, p. 168
Paradentiscutata B.T. Goto, Oehl & G.A. Silva, p. 122
Paradentiscutata bahiana Oehl, Magna, B.T. Goto & G.A. Silva, p. 123
Paradentiscutata maritima B.T. Goto, D.K. Silva, Oehl & G.A. Silva, p. 126
Passalora macfadyenae Meir. Silva, O.L. Pereira & R.W. Barreto, p. 187

Penicillium guanacastense K.G. Rivera, Urb & Seifert, p. 324
Penicillium mallochii K.G. Rivera, Urb & Seifert, p. 322
Phlebopus spongiosus Pham & Har. Takah., p. 28
Pleurophragmium indicum M.A. D'Souza & Bhat, p. 477
Prospodium macfadyenae Meir. Silva, O.L. Pereira & R.W. Barreto, p. 191
Pseudobaeospora deckeri C.F. Schwarz, p. 466
Pseudocercosporella stellariicola M.J. Park, J.H. Park & H.D. Shin, p. 270
Psilocybe thaiaerugineomaculans Guzmán, Karunarathna & Ram.-Guill., p. 69
Psilocybe thaicordispora Guzmán, Ram.-Guill. & Karunarathna, p. 72
Psilocybe thaiduplicatocystidiata Guzmán, Karunarathna & Ram.-Guill., p. 74
Psilocybe thaizapoteca Guzmán, Karunarathna & Ram.-Guill., p. 77
Radulomycetopsis Dhingra, Priyanka & J. Kaur, p. 133
Radulomycetopsis cystidiata Dhingra, Priyanka & J. Kaur, p. 135
Remototrachyna aguirrei (Sipman, Elix & T.H. Nash) Flakus, Kukwa & Sipman, p. 161
Remototrachyna consimilis (Vain.) Flakus, Kukwa & Sipman, p. 161
Remototrachyna singularis (Hale) Flakus, Kukwa & Sipman, p. 162
Remototrachyna sipmaniana Kukwa & Flakus, p. 162
Skeletocutis bambusicola L.W. Zhou & W.M. Qin, p. 346
Skeletocutis subodora Vlasák & Ryvarden, p. 228
Solicorynespora cryptocaryae Jian Ma & X.G. Zhang, p. 98
Solicorynespora ligustri Jian Ma & X.G. Zhang, p. 96
Solicorynespora machili Jian Ma & X.G. Zhang, p. 97
Sporidesmium antidesmatis Jian Ma & X.G. Zhang, p. 19
Sporidesmium liquidambaris Jian Ma & X.G. Zhang, p. 18
Sporidesmium machili Jian Ma & X.G. Zhang, p. 21
Terriera petrakii C.L. Hou, p. 332
Terriera rotundata C.L. Hou, p. 330
Tuber microspermum L. Fan & J.Z. Cao, p. 392
Tuber microspiculatum L. Fan & Yu Li, p. 392
Volvariella sathei Senthil., Rahul Sharma & S.K. Singh, p. 470
Xylaria atroglobosa H.X. Ma, Lar.N. Vassiljeva & Yu Li, p. 382
Zelodactylaria A.C. Cruz, Gusmão & R.F. Castañeda, p. 242
Zelodactylaria veticillata A.C. Cruz, Gusmão & R.F. Castañeda, p. 242

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